Universidade de Lisboa

Faculdade de Farmácia

Dissertation

Range and output simulation for elemental impurities in drug products

Rodrigo Filipe Teixeira da Silva

Dissertation supervised by Professor Rui Loureiro and co-supervised by Professor Dr. Joana Marques Marto.

Master degree in Pharmaceutical Engineering

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“Imagination is more important than knowledge. For knowledge is limited to all we now know and understand, while imagination embraces the entire world, and all there ever will be to know and understand.”

(Albert Einstein)

ABSTRACT

This master's degree in Pharmaceutical Engineering was developed at the Faculty of Pharmacy of the University of Lisbon (FFUL) in partnership with the Laboratório Edol - Produtos Farmacêuticos, S.A.

This project is based on a strong innovative component, since the control of elemental impurities has only applied to oral, parenteral and inhalation pharmaceutical forms. Therefore, the main objective of this project is perform a risk assessment for the control of EI in ophthalmological drugs of chronic, acute and sporadic use. To do this, it was necessary to carry out a study, as detailed as possible, of the entire process and components involved in the production of the three drugs products, making it essential to collect all process, product and use parameters.

After an in-depth knowledge of all those involved in the manufacturing process of each of the ophthalmological drugs under study, a Risk Assessment methodology was developed based on the principles of ICH Q3D. In this methodology the various calculation options available in the ICH Q3D are tested and the control of the levels of Impurities is performed using the Permitted Parental Daily Exposure made available in the ICH Q3D, since due to the lack of information, it was not possible to calculate the PDEs of the ophthalmic route.

With this project, it was possible to conclude that EI is commonly present in the most materials that come in direct contact with the drug and may affect the drug's efficacy and the health of the patient. It was also possible to verify that the lack of data was the greatest condition in the accomplishment of the same one, since it was necessary to resort to data of bibliography that represent scenarios quite maximized but still l most of the sources of elemental impurities are considered **negligible**, since the levels presented are much lower than the CL. Therefore, the Risk Assessment approach is an adequate strategy to control EI in ophthalmic drug products.

The goal of this project has been successfully achieved, and a risk assessment methodology for ophthalmic products is now available.

Key words: Elemental impurities, Eye drops solution Determination of Elemental Impurities, Simulation, Risk Assessment

RESUMO

Este projeto de mestrado em Engenharia Farmacêutica foi desenvolvido na Faculdade de Farmácia da Universidade de Lisboa (FFUL) em parceria com o Laboratório Edol - Produtos Farmacêuticos, S.A.

As impurezas elementares são contaminantes que podem ser oriundos da adição intencional de catalisadores, na síntese da substância medicamentosa, ou podem surgir naturalmente devido à contaminação ambiental dos excipientes ou substância ativa que compõem a formulação do medicamento.

As interações existentes entre os equipamento e todos os materiais que contactam diretamente com o produto durante a sua produção, incluindo os materiais de armazenamento, que por norma no caso de colírios são feitos de polímeros, são outro fator a ter em consideração numa avaliação de risco devido aos longos tempos de exposição do produto a estes materiais. Assim sendo, e sabendo que as impurezas elementares não oferecem qualquer benefício terapêutico e que por outro lado podem afetar negativamente o comportamento toxicológico do fármaco é extremamente importante controlar os seus níveis a fim de se garantir que estes se encontram dentro dos limites aceitáveis. O controlo das impurezas elementares era efetuado recorrendo ao método colorimétrico, no entanto, devido às suas limitações, e com o objetivo de se harmonizar os requerimentos técnicos para a regulação de impurezas elementares em produtos farmacêuticos de três regiões (Europa, Japão e Estados Unidos) a ICH iniciou, em 2009, a Q3D. Este documento classificou as impurezas elementares em 3 classes e estabeleceu os PDE para os 24 elementos. No entanto, este guia apenas apresenta os PDE's para as vias de administração oral, parentéricas e inalatórias, ficando por estabelecer para as restantes vias. Sugere ainda que o controlo das impurezas elementares seja efetuado recorrendo aos princípios descritos na ICH Q9 assim sendo, este projeto assenta numa forte componente inovadora, dado que o principal objetivo deste projeto é a realização de uma avaliação de risco para o controle de impurezas elementares em medicamentos oftalmológicos de uso crónico, agudo e excecional. Para que tal seja exequível, foi necessário efetuar-se um estudo, o mais detalhado possível, de todo o processo e intervenientes envolvidos na produção dos três medicamentos, tornando-se essencial a recolha de todos os parâmetros de processo, produto e utilização.

Após um conhecimento aprofundado de todos os intervenientes no processo de fabrico de cada um dos medicamentos oftalmológicos em estudo, desenvolveu-se, baseada nos princípios da ICH Q3D, uma metodologia de *Risk Assessment*. Nesta metodologia são testadas as várias opções de cálculos disponibilizadas na ICH Q3D e o controle dos níveis de Impurezas é realizado recorrendo aos PDE's parentéricos disponibilizados na ICH Q3D, dado que devido à inexistência de informação, não foi possível efetuar o cálculo dos PDE's da via oftalmológica e a absorção medicamentosa nesta via é maioritariamente por via parentérica. A metodologia apresentada contempla 7 etapas:

- **Etapa 1** – Identificação das fontes de impurezas elementares – Nesta etapa é realizado um estudo aprofundado de todos os componentes que contactam diretamente com o produto durante a sua produção, pretende-se obter uma lista exaustiva de todos os intervenientes a fim de se obter uma perspetiva global dos

potenciais contaminantes do medicamento. Para que tal seja exequível, por norma recorre-se a um diagrama Ishikawa.

- **Etapa 2** – Avaliação da contribuição da substância ativa e excipientes para a presença de impurezas elementares no produto final – Após a identificação da substância ativa e respetivos excipientes, foi necessário obter informação relativa à presença de impurezas elementares nos mesmos. Assim sendo, contactou-se os respetivos fornecedores que, quando tinham a informação disponível, enviaram os respetivos dossiers com os elementos presentes nos seus produtos e as respetivas concentrações. No caso de inexistência de resposta foi decidido assumir o *worst-case scenario*, isto é, que cada impureza assumiria a concentração permitida disponibilizada pela ICH Q3D. Após se obter esta triagem, ficou decidido quais os elementos a incluir nesta avaliação, nos casos em que o fornecedor não disponibilizou dados, assumiu-se que para esse produto as impurezas elementares não teriam sido intencionalmente adicionadas e por esse motivo a avaliação de risco foi realizada segundo as recomendações da ICHQ3D para elementos a serem considerados na avaliação de risco para a via de administração parentérica. De seguida comparou-se as concentrações de cada impureza elementar com as concentrações limite obtidas pelas opções de cálculo 1, 2A e 2B, disponibilizadas pela ICH Q3D.

- **Etapa 3** – Avaliação da contribuição do equipamento de fabrico para a presença de impurezas elementares no produto final – O equipamento que contacta diretamente com o produto durante toda a fase de produção, incluindo o embalamento, pode constituir sérios riscos para a presença de impurezas elementares. Assim sendo, foi efetuado um levantamento criterioso de todo o equipamento, para os 3 produtos em teste, com o objetivo de se obter a sua composição e assim entender quais os potenciais elementos contaminantes. Do contacto com os fornecedores apenas se obteve os certificados analíticos de qualidade, tendo sido necessário recorrer-se à pesquisa bibliográfica para se obter a constituição do aço inox. Em termos de avaliação de risco, optou-se por usar uma abordagem conservativa em que se assume que por cada lote de fabrico migra 0.5 g/metal para o produto final. Assim, a contribuição do equipamento para a presença de impurezas elementares foi realizada para o lote de fabrico mais pequeno, de entre os produtos em tese, com o objetivo de se obter o *worst-case scenario*.

- **Etapa 4** - Avaliação da contribuição dos filtros para a presença de impurezas elementares no produto final – Após o levantamento dos filtros utilizados durante o processo de fabrico contactou-se os fornecedores a fim de se obter os elementos presentes nos mesmo, dado que não se obteve resposta, recorreu-se à pesquisa bibliográfica para se estimar os elementos e as respetivas concentrações. Salienta-se que as concentrações obtidas estão bastante sobrestimadas, pois as condições

onde são realizados os testes de extração são bastante mais agressivas que as próprias características do produto.

- **Etapa 5** - Avaliação da contribuição do sistema de tratamento de água para a presença de impurezas elementares no produto final – A água é o elemento mais comum na maioria das preparações farmacêuticas, especialmente nos colírios, e é facilmente contaminado. Assim sendo, foi realizado um estudo de todo o sistema de tratamento de água, permitindo identificar todos os materiais que contactam diretamente com a água. Após o contacto com os fornecedores verificou-se que a informação obtida era bastante escassa tendo sido necessário recorrer à pesquisa bibliográfica.

- **Etapa 6** - Avaliação da contribuição do sistema de acondicionamento para a presença de impurezas elementares no produto final – Os sistemas de acondicionamento são considerados uma das maiores fontes de impurezas elementares, dado que o tempo de residência do produto é bastante elevado. Tendo em conta este facto, é extremamente importante efetuar um levantamento do material que constitui este sistema. No caso dos produtos em teste estes apenas contactam com um tipo de polímero, tendo sido o próprio fornecedor a realizar os testes para a presença de impurezas elementares e a disponibilizá-lo.

- **Etapa 7** – Resumo da Avaliação de Risco – Nesta etapa foi decidido estudar-se 3 cenários, para se obter uma perspetiva diferente das várias possibilidades em estudo. Esta etapa permite ao aplicante decidir se é necessário recorrer a ações de correção, recorrendo para isso aos princípios descritos na ICH Q3D.

Com a implementação desta metodologia foi possível verificar, que embora os cenários em teste estejam bastante maximizados, que a análise de risco é uma abordagem perfeitamente adequada para a quantificação destes contaminantes, pois as concentrações obtidas são bastante abaixo das concentrações limite.

A realização deste projeto científico permitiu ainda verificar que a ICH Q3D apresenta algumas limitações, nomeadamente ao nível das opções de cálculo disponibilizas, pois estas deveriam ter em consideração as vias de administração e a forma farmacêutica em estudo. Assim sendo, o objetivo deste projeto foi alcançado com sucesso, e uma metodologia de avaliação de risco para produtos oftalmológicos encontra-se agora disponível.

Palavras-Chave: Impurezas Elementares, Colírios, Determinação de Impurezas Elementares, Simulação, Avaliação de Risco

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LIST OF ABREVIATIONS

AAS	**Atomic Absorption Spectrometry**
Ag	**Silver**
Al	**Aluminium**
API	**Active Pharmaceutical Ingredient**
As	**Arsenic**
Au	**Gold**
B	**Boron**
Ba	**Barium**
Bi	**Bismuth**
BP	**British Pharmacopeia**
Ca	**Calcium**
Cd	**Cadmium**
CL	**Concentration Limit**
Co	**Cobalt**
Cr	**Chromium**
EI	**Elemental Impurities**
EMA	**European Medicines Agency**
Fe	**Iron**
GR-AAS	**Atomic Absorption Spectrometry including graphic furnace**
HDPE	**High Density Polyethylene**
Hg	**Mercury**
ICH	**International Conference on Harmonization**
ICP	**Inductively Coupled Plasma**
ICP-MS	**Inductively coupled plasma mass spectrometry**
ICP-OES/AES	**Inductively Coupled Plasma Optical Emission Spectrometry**
Ir	**Iridium**
JP	**Japanese Pharmacopoeia**
K	**Potassium**
LDPE	**Low Density Polyethylene**
Li	**Lithium**
LO(A)EL	**Low-Observed-Effect Level**
MDD	**Maximum Daily Dose**

Mg	**Magnesium**
Mn	**Mn**
Mo	**Molybdenum**
Na	**Sodium**
Ni	**Nickel**
NO(A)EL	**Non-Observed-Effect Level**
Os	**Osmium**
Pb	**Lead**
Pd	**Palladium**
PDE	**Permitted Daily Exposure**
Ph.Eur	**European Pharmacopoeia**
Pt	**Platinum**
QRM	**Quality Risk Management**
Rh	**Rhodium**
Ru	**Ruthenium**
Sb	**Antimony**
Se	**Selenium**
Sn	**Tin**
Tl	**Thallium**
USP	**United States Pharmacopoeia**
V	**Vanadium**
W	**Tungsten**
Zn	**Zinc**

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CHAPTER 1 – Introduction

Elemental impurities (EI) are external entities to the drug product that may arise from a variety of sources. They can be added during the production of raw material, occur naturally by environmental contamination or result from contamination by the equipment and packaging systems. Because they have no therapeutic benefit and are associated with drug safety and efficacy problems, they should be controlled [1–3].

The implementation of the ICH Q3D guideline, which establishes the permitted daily exposure (PDE) of each element for the oral, inhalation and parenteral routes, has led the pharmaceutical industry to focus on this theme, suggesting the control of these impurities [2,4]. The objective of this project is to realize a Risk Assessment to implement this guideline to three products for ophthalmologic application and for chronic, acute and sporadic use, thus making it necessary to obtain an EI control methodology for the respective route of administration, to obtain the maximum level of elemental impurities in products [2].

This project is divided in 6 chapters, the next chapter – LITERATURE OVERVIEW - has the purpose of giving the background required to understand the issues addressed in the later chapters. Therefore, the focus is mainly on EI and its regulation, and on the principles of Quality Risk Management (QRM). Following this, the chapter – MATERIALS AND METHODS – are described the materials and methods used to perform the experimental procedures related to the performance of the risk assessment are described. This chapter is focus on data processing, methodology for obtaining the PDE's for the ophthalmic route, on options for control the levels of EI in active substance and excipients and finally in the methodology proposed for the implementation of the Risk Assessment.

In chapter – RESULTS AND DISCUSSION OF RESULTS - presents the results obtained for the three products applying in a detailed way the methodology developed. These results are duly discussed during this chapter. Finally, in Chapter – CONCLUSIONS -, the conclusions reached in this study are presented and on Chapter – FURTHER CONSIDERATIONS – are given future work perspectives, allowing the continuity of the work presented here.

CHAPTER 2 – Literature Review

2.1 Regulation of Impurities

The impurities in drug products often possess unwanted pharmacological–toxicological effects and do not provide any therapeutic benefit to the patient, their levels in the drug product should be controlled within acceptable limits [5][6].

In 1998, the European Medicines Agency (EMA), initiate the development of a guideline on residual catalysts in pharmaceuticals. The goal of this guidance is to establish limits for the control of 14 metal catalyst or metal reagents on toxicological safety assessment [7][8]. In 2008, The EMA Guideline on Specification Limits for Residues of Metal Catalysts or Metal Reagents was officially implemented for new drug products. This guidance introduces the principle of the mass-based PDEs to establish permissible exposures in drug products rather than concentration limits in drug product. The PDEs in this guideline were based on assessments of toxicological data on individual metals [7].

The EMA guideline clusters the 14 metal residues into three classes. Metals with significant toxicity, including human carcinogens, are placed in Class 1. This class are further subdivided into three subclasses called 1A, 1B and 1C. The class 1A include Platinum (Pt) and Palladium (Pd). Iridium (Ir), Osmium (Os), Rhodium (Rh) and Ruthenium (Ru) elements are placed in Class 1B. Class C elements are Molybdenum (Mo), Nickel (Ni), Chromium (Cr) and Vanadium (V). In Class 2 are placed metals with a low safety concern and which includes Cupper (Cu) and Manganese (Mn). Metals with no significant toxicity are grouped in Class 3. This class include Iron (Fe) and Zinc (Zn) [9]. **Figure 1** indicates classification of elements by EMA.

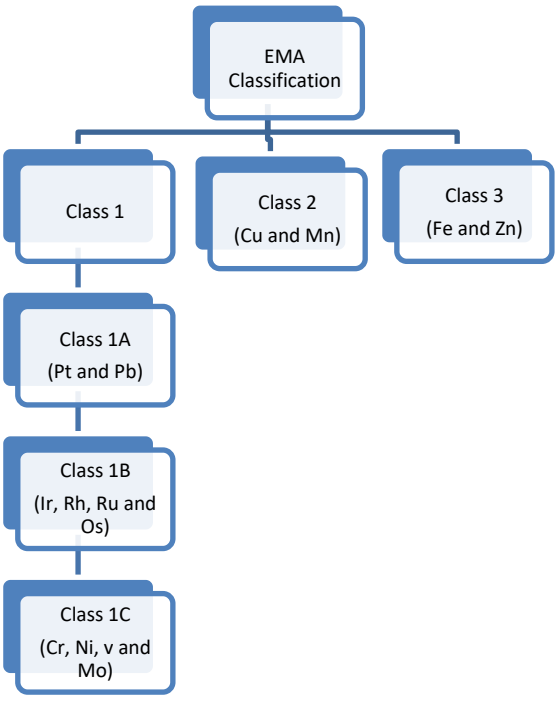

Figure 1. EMA classification of EI. Adapted (9).

Later in 2008, the USP proposed introduce two new chapters, chapter <232>, which would establish safety based limits on elemental impurities in pharmaceutical products, and <233> which would establish appropriate criteria in methods for elemental analysis, to replace the old colorimetric method <231> These chapters are official implemented in February 2013 [7].

In 2009, with the scope of harmonizing technical requirements for elemental impurities in pharmaceutical products across three regions (Europe, Japan, and United States), the International Conference on Harmonization (ICH) initiated the Q3D expert working group. This working group, like in EMA guidance and USP chapters, try to set maximum PDE's for elemental impurities in pharmaceutical products based on assessment of existing toxicological data for oral, parenteral and inhalation routes of administration. In June 2013, ICH Q3D reached step 2 and was published for a public review. The step 4 reaches in November 2014 and the USP Expert Panel on Elemental Impurities align Chapters <232> and <233> with Q3D [7].

This council group propose classify 24 elemental impurities in three classes, based on their toxicity (PDE) and likelihood of occurrence in the drug product for inhalation, parenthetic and oral route. The probability of occurrence of each EI depends on factors such as: likelihood of use in pharmaceutical process, probability of being a co-isolated impurity with other elemental impurities in materials used in pharmaceutical process, and the observed natural abundance and environmental distribution of the element [2][6]. The elements are dived in three classes and two subclasses **(Table 1)**. The class 1 includes elements that are human toxicants and recommend evaluate them during the risk assessment. Their presence normally comes from used materials, such as mined excipients. Because your toxicity, the use of this elements needs to be limited or excludes in the manufacture of pharmaceuticals. In Class 2, the probably of occurrence the impurities in drug product are subdivided in Class 2A, 2B and 2C. The elements in Class 2A have more probably of occurrence in drug product than Class 2B elements, for this reason, these elements should be included in the risk assessment while Class 2B elements need not to be considered during the risk assessment, because they have low potential to be co-isolated with other materials. In the Class 3, are included the elements with low toxicity but they may require consideration for the risk assessment for inhalations and parenteral routes [2].

This guidance still suggests some other elemental impurities, but because their low inherent potential toxicity and/or differences in regional regulations the PDEs are not established. The elements considered are: Aluminium (Al), Boron (B), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Tungsten (W) and, Zinc (Zn) [2][6].

Table 1. ICH Q3D Classification of elemental Impurities and the need for Risk Assessment considering the route of administration. Adapted from (2).

Class	Elements	Characteristics	Risk Assessment			
			If intentionally added (all routes)	If not intentionally added		
				Oral	Parental	Inhalation
1	Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb)	Toxic elements that have limited or no use in the manufacture of pharmaceuticals, require risk assessment across all potential sources of EI and routes of administration.	Yes	Yes	Yes	Yes
2A	Cobalt (Co), Nickel (Ni), and Vanadium (V)	High probability of occurrence in drug product, require risk assessment across all potential sources of EI and routes of administration.	Yes	Yes	Yes	Yes
2B	Silver (Ag), Gold (Au), Iridium (Ir), Osmium (Os), Palladium (Pd), Platinum (Pt), Rhodium (Rh), Ruthenium (Ru), Selenium (Se), and Thallium (Tl)	Reduced probability of occurrence in drug product, excluded from the risk assessment unless they are intentionally added during the manufacture of drug product.	Yes	No	No	No
3	Barium (Ba), Chromium (Cr), Lithium (Li), Molybdenum (Mo), Antimony (Sb), and Tin (Sn).	Low toxicities by the oral route of administration but may require consideration in the risk assessment for inhalation and parental routes.	Yes	No	Yes, for Cu, Li, and Sb, no for Ba, Cr, Mo, and Sn	Yes

This guidance also suggests that EI in the drug should be controlled using the principles of Quality Risk Management, described in ICH Q9: “Quality Risk Management”. With this approach, the applicant can identify/analyse the risk, evaluate (compare levels with the PDE’s) and controls as required [(2)]. But this guidance does not provide any specific tool to perform this[(2)].

The ICH guideline also provides a number of specific control options, to ensure that the concentration of elemental impurities in drug product or their components not exceed the PDEs. **Option 1:** Common permitted concentration limits of elements across drug product components for drug products with daily intakes of ≤10 g, providing a simplified approach to the PDE calculations. The option assumes that elemental impurities identified in the risk assessment (the target elements) are present in all components of the drug product [(2)].

Option 2a: As per Option 1, except that the calculation is modified to include the specific product dose [(2)].

Option 2b: Permitted concentration limits of elements across drug product component materials for a product with a specified daily intake. This option allows the applicant to establish limits in terms of permitted concentrations for each individual component based on the distribution of elements in the components, i.e., it permits higher concentrations of specific elements in some components provided that for each element the total amount of the elemental impurity in the final drug product does not exceed the permitted limit [(2)].

Option 3: Finished Product Analysis. The concentration of each element can be measured in the drug product [(2)].

This guideline covers all elemental impurities, including those arising from natural sources and/or process impurities. In contrast, the earlier EMA guideline specifically focused on metal catalysts / metal reagents.

The ICH Q3D Step 5 has been effect for new marketing authorisations since June 2016 and for existing authorised medicinal products since 2017 [(10)]. The new USP General Chapters USP<232> 'Elemental Impurities – Limits', USP<233> 'Elemental Impurities – Procedures' and USP<2232> 'Elemental Contaminants in Dietary Supplements has implemented in 1 January 2018 [(11)(10)].

2.2 Impurities

According to ICH, an impurity in a drug substance is defined as "Any component of the drug product which is not the chemical entity defined as the drug substance or an excipient in the drug product" (ICH Q6A: Specifications) [(12)]. In pharmaceutical world, an impurity are considered an external compound besides drug substance, or excipients, which arise out of synthesis or the unwanted chemicals that remains with the Active Pharmaceutical Ingredient (API) [(13)].

Because pharmaceutical impurities are also referred to as "drug-related substances" they can arise from a variety of origins including starting materials, reaction by products generated during synthesis of drug substances, intermediates, degradation products formed during the formulation manufacture process and/or storage of formulated products, reagents, ligands and catalysts [(14)(15)]. However, impurities can have safety and efficacy implications and are therefore the subject of considerable attention by both the manufacturer (industry) and regulatory [(15)].

The safety of a drug product, or a dosage form, depends not only on the toxicological properties of the drug substance but also on the properties of those pharmaceutical impurities [(14)]. The presence of impurities in pharmaceuticals, even in small amounts, is a concern, not only because some contaminants are inherently toxic, but because they may adversely affect the drug stability, efficacy and, self-life of the drug product, or may cause unwanted side-effects [(16)(17)(18)].

While the use of pharmaceuticals is always a balance of risks and benefits, the same is not true for impurities in pharmaceuticals, because impurities convey only risk and not have any therapeutic benefit to the patient, they are expected to be known and rigorously managed [(19)(3)]. Monitor and control of impurities generally gives assurance of the quality and safety of a drug, but the control of pharmaceutical impurities are considerate a critical and challenging issue by pharmaceutical industry and regulatory agencies, because they try to create norms to assure that the level drug impurities does not reach high risk values [(5)(20)(19)].

Impurities in drug product can be dived in two types: Impurities associated with the API and Impurities formed during the formulation and/or aging or that are related to the formulated forms [17].

The impurities associated with the API can be classified in three categories and different names can be adopted to classify these impurities, but according ICH guidelines, impurities can be classified in organic, inorganic and residual solvents [17][13][16].

Organic impurities are commonly found in API's and can arise during the manufacturing processes, including synthesis steps, and/or packing materials [16][21][22]. They may be identified or unidentified, volatile or non-volatile [17]. This type of impurities can be called "process and drug related" and can include: [21][22]

- Starting materials or intermediates
- By-products of the synthesis:
- Degradation products
- Material using during the synthesis like reagents, ligands and catalysts [21][22].

Inorganic impurities are associated to manufacturing process and normally they are known and identified [16][21]. They can include:

- Reagents, ligands, and catalysts
- Heavy metals
- Inorganic salts
- Other materials like filters aids and charcoal [16][21][22].

Residual solvents are organic volatile chemicals used in the manufacturing process of drug substances or excipients, or generated during the production [16]. Normally this type of impurity can't be totally removed by practical manufacturing techniques [21]. The residual solvents are classified into three classes, depending on the level of risk they present to human health [16]:

- **Class 1 solvents**: Identifying with human carcinogens, strongly suspect human carcinogens, and environmental hazards. This class is to be avoided.
- **Class 2 solvents:** Include non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity. Solvents suspected of other significant but reversible toxicities. The use of this solvents is to be limited.
- **Class 3 solvents:** Solvents with low toxicity to human health [21].

2.3 Heavy metals to Elemental Impurities

Heavy metals are natural compounds of the Earth's crust and they cannot be degraded or destroyed. This components can enter in human body via food, drinking water, cigarettes and air [23]. But, in the past, the term "Heavy Metals" was erroneously used in literature to refer a group of metals, metalloids, and some non-metals which had some toxicity and were associated to contamination of pharmaceuticals. This term has no basis connection with toxicological or chemical data and suggest that all compounds of the same element (organic and/or inorganic) have the same physical, chemical and toxicological properties, which is not true for the most elements. In addition, heavy metals refer to any metallic chemical element that has relatively high density and is toxic at low concentrations. For the most pharmacopoeias the term heavy metals are "metallic impurities that are coloured by 7 sulphide ion, under the specified test conditions" but this designation is limited because this term include other elements and only a specific group of elements respond to this test, in addition some relevant elements with toxicological relevance are not covered by this test. To solve this problem, a new designation for the term heavy metals was created and the term IE comes as a new designation for this group of elements [24].

2.4 Elemental Impurities

Are defined as elements that are found in the environment and that can be used or introduced in the manufacture of drug substances or excipients [25][8]. This term is adopted an alternative to the ill-defined term heavy metals and include various transition metals and metalloids [26].

This metallic impurities can be present in pharmaceuticals from several sources and via a number different means [27][3]. They can be present in the drug product by intentional addition in chemical synthesis (metal catalysts or metal reagents),or be present by as a contaminant resulting the interactions with the process equipment and piping, raw materials, water, the environment, cleaning solvents and storage systems that contact directly with the drug product [6][26][27][28].

The presence of this elemental (inorganic) impurities in finish drug products, even in small amounts, may influence the safety and efficacy of the drug product [17]. Their levels in the drug product should be known and controlled with the acceptable limits, because these contaminates not provide any therapeutic benefit and pose some risk to the patient health due to toxicological effects [6].

Some elements classified as EI, like zinc, copper, chromium, iron, and manganese are considerate to be essential to the human nutrition, as they play critical roles in the structures of proteins and the activities of enzymes, but only in trace and ultra-trace levels [5][27][29]. On the other hand, some elements such arsenic, mercury and lead, are known to be quite toxic to human beings [5]. According this, the level of elemental impurities in drug products should be managed, known and low [3].

2.4.1 Sources of Contamination

Elemental Impurities can be present in final drug product by intentional addition of the elements in the production process, such reagents and catalysts or can result of the elements cannot completely removed from the API synthesis [(2)(25)]. There are also other sources of contamination related to the materials used in equipment and all surfaces (generally metals) that directly contacts with API or drug product. The incorporation of elemental impurities in drug product may be related to phenomena such as corrosion extraction/leaching, or delamination, like it was verified in contamination by aluminium from glass, zinc from plastic and rubber materials used in container closure systems [(24)].

The container closure system is another source of elemental impurities because the elemental entities present in the materials of construction of this systems, that contact directly with the drug product, may leach to the during the time that the drug product is in contact with the polymers, and these leached elemental impurities become elemental impurities in drug product [(3)].

Water is another source of contamination of the drug product because it is widely used in many pharmaceutical process, from synthesis to the production of pharmaceutical dosage forms and if any problem related to the quality of this component are not detected this can contaminate the drug product. According this, and because these contaminations are very difficult to detect, it is very useful to choose an appropriate Water Treatment System. To control the level of contamination of the water, is strongly recommended to determine EI level in all pharmacopoeias [(24)]. In **Figure 2**, are identified the potential sources of metallic impurities present in production process of pharmaceuticals.

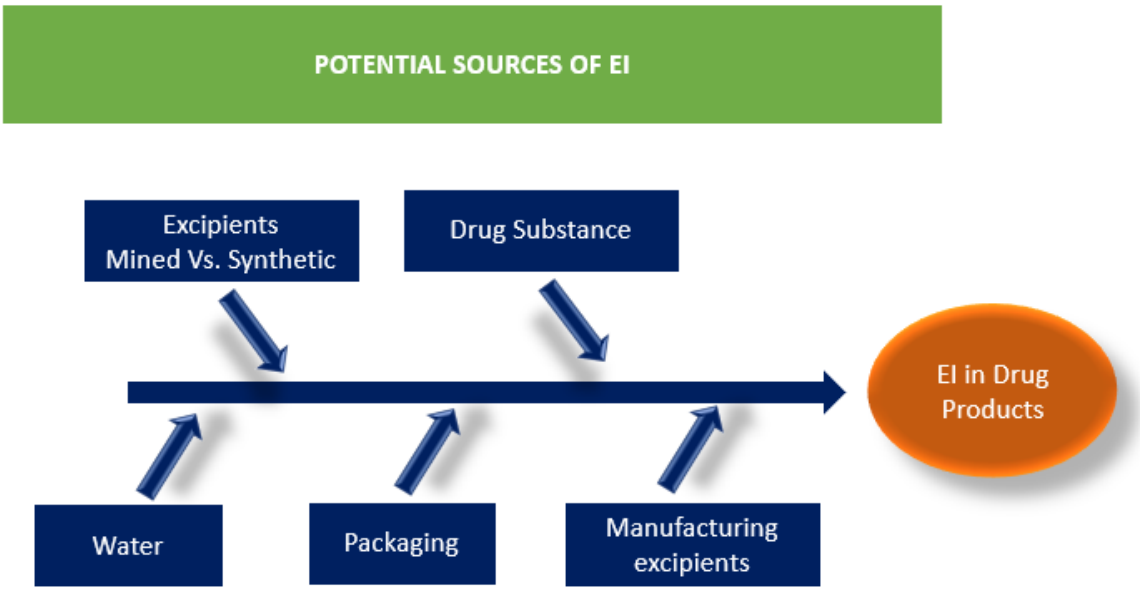

Figure 2. Potential Sources of metallic impurities during the production process of pharmaceuticals. Adapted (28).

Knowledge about the presence, level and “leachability” of elemental entities in polymers used in manufacturing and packaging systems is relevant to understanding how manufacturing and packaging systems contribute to a drug product’s total elemental impurity burden [3].

2.4.2 New methods used to determine EI in drug product

The pharmaceutical industry is subject to a high level of regulation, leading to the development and manufacture of drugs controlled by government agencies through a set of laws and guidance documents. The main propose of regulations is to ensure quality, efficacy and safety of drugs [8]. The world regulatory agencies require the control and monitoring of the toxic elements to acceptable levels in pharmaceutical industry [11].

The acceptable levels of heavy metals in pharmaceuticals usually are defined by the regulatory agencies and controlled by limit tests. These limit tests are regulated by pharmacopoeias and permit ensure the absence that inorganic contaminants in drug products [5][8]. Due this, the USP, British Pharmacopoeia (BP), European Pharmacopoeia (Ph.Eur) and Japanese Pharmacopoeia (JP) have developed chapters to propose collective monitoring of total metal content in drugs and drugs substances [5][8][30]. These pharmacopoeia methods include this elements: As, Cd, Cu, Sn, Sb, Pb, bismuth (Bi), Ag, Hg and Mo and the methodology is similar in all pharmacopoeias, involve the precipitation of metal sulfides from a weak acid media and the colours of metal sufides range from white to yellow, orange, brown and black, this precipitate is compared by visual comparison with a 10 ppm lead sulphide reference standard (dark brown) [8][30][5]. This test has been in place for over 100 years, and different names are used by pharmacopoeias to designate this method. In USP this test is called Chapter <231>: Heavy Metals Analysis and Ph.Eur General Chapter 2.4.8 Heavy metals [8][27].

This colorimetric method have some significant limitations, that including non-specific method, less sensitive, time consuming and less accurate [5]:

- Subjective and difficult visual exam of the sample solution compared to standard solution.
- The result of this method is seldom reliable and reproductible, because the formation of the sulfides is affected by sample matrix and hence.
- Only applied to elements that form coloured sulfide precipitates, applicable to ten elements (Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu and Mo).
- Non-selective method, which means it cannot distinguish between elements with high and low toxicity.
- Requires large sample amounts for low detection levels (for example a minimum of 2 g sample is required for a detection limit of 10 ppm).
- Time consuming and require many workers.
- The sample preparation can be leading to the loss of volatiles elements, because it occurs in a furnace at 600 degrees. It is mean that the procedure does not provide real quantitative results. (example: mercury in solid samples is not recovered at all) [8][9][31].

According to the limitations of the old colorimetric method, the Ph.Eur, USP and ICH have introduced new changes in regulation of elemental (inorganic) impurities, motivating an increase of the interest of determination of elemental impurities in pharmaceutical products [2][28][24].

With this new changes, the wet chemical and colorimetric test have been replaced with instrumental methods that provide specific, quantitative determination of individual elemental impurities in drug products and ingredients and new analytical methods are proposed (32). The USP replace the old method with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES/AES) and the Ph.Eur provides a several general chapters for the analysis of elemental impurities using the analytical methods Atomic Absorption Spectrometry (AAS) , including graphic furnace AAS (GF-AAS), Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-OEA/AES) and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) [11][8].

Determination of EI by ICP-based methods in pharmaceuticals is increasing, like we can observe in **Figure 3**. This increasing may be justified by the multielemental capability of plasma-based instruments, especially when compared with absorption spectrometry (AAS) [24].

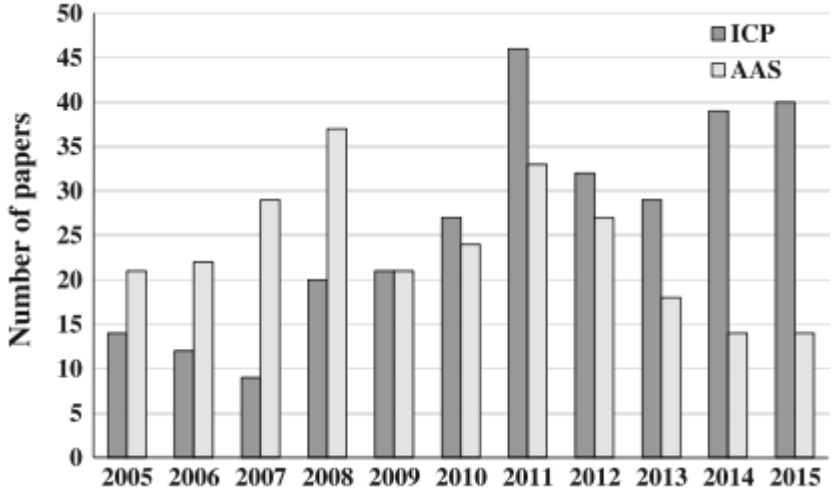

Figure 3. Articles published from 2005 to 2015 (up to October 2015) regarding to the use of inductively coupled plasma (ICP)-based and atomic absorption spectrometry (AAS) methods for the determination of elements elemental impurities in pharmaceutical products. Data received from: (24).

In **Figure 3** it is possible to verify that from the year 2008 to the year 2009, the interest by the ICP methods had a great prospect because two important documents from EMA and USP, both published in 2008, that refer to the evaluation of elemental impurities certainly contributed to increase the interest of this techniques. These documents alerted the community to the importance of establishing limits of concentration for EI and given the ability of these equipment's to carry out analyses of multielements, these techniques were considered a valid option in the determination of EI. In 2010, the USP and BP create two general chapters to determine EI in APIs and this chapters are published in 2012 in EP. In these pharmacopoeias, the use of spectrometry techniques, including ICP-MS and ICP-OES, is recommended [24].

The main advantages and limitations of using ICP methods are described, covering the applications reported in the literature mainly for active substance, raw materials, and pharmaceutical dosage forms [24]. These advantages include:

- Ability to identify and quantify all elements with exception of Aragon.
- Suitable for all concentrations from ultra-trace levels to major components because many wavelengths of varied sensitivity are available for determination of any one element.
- Detection limits are generally low for most elements with a typical range of 1-100 g/L.
- Perform multielemental analysis quite and rapidly, a complete multielemental analysis can be undertaken in a period as short as 30 seconds and consuming only 0.5 ml of sample solution [33].

2.5 . Quality Risk Management

Quality Risk Management (QRM) is a systematic process for the assessment, control, communication and review of risks to the quality of the drug (medicinal) product across the product lifecycle [34][35]. QRM concept depends upon the understanding of terms 'Quality' and 'Risk'. The term Quality means "The degree to which a set of inherent properties of a product, system or process fulfils requirements" (ICH Q9) and as per ISO/IEC Guide 51, the term Risk means "The combination of the probability of occurrence of harm and the severity of that harm" [35].

Quality Risk Management, as described in ICH Q9, can be used in a variety of activities including assessing options for the design of the manufacturing process, assessing quality attributes and manufacturing process parameters, and increasing the assurance of routinely producing batches of the intended quality [36]. It can provide a proactive approach to identifying, scientifically evaluating and controlling potential risks to quality. It facilitates continual improvement of process performance and product quality throughout the product lifecycle [37].The QRM process involves:

- Hazards (sources of harm) that can adversely influence drug quality characteristics.
- Extent of harm.
- Sub processes critical for quality [35].

A model for QRM outlined in the diagram **(Figure 4)**. Other models could be used [34].

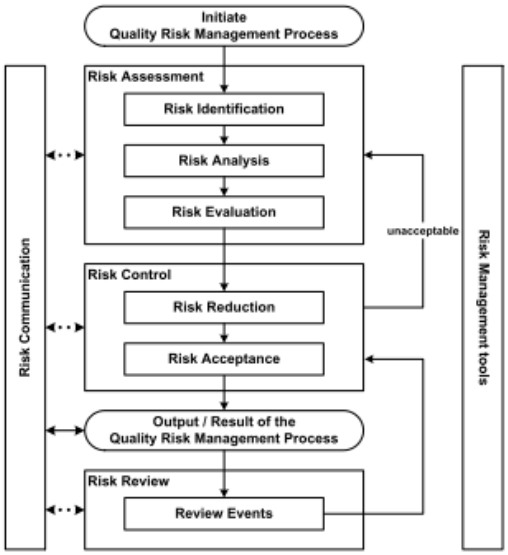

Figure 4. Overview of a typical quality management process (34).

2.5.1 Risk Assessment process

Risk Assessment, according to the principals described in ICH Q9: Quality Risk Management, has a part of a quality Risk system **(Figure 4)** and consists of the identification of hazards and the analysis and evaluation of the Risk associated with exposure to those hazards. According this, Risk Assessment is an simple way to minimize the process Risk, which should include a multidisciplinary expertise group and to begin with this approach it is necessary to have an well-defined problem description [38][34]. To do this, there are three fundamental questions:

- What can go wrong with the process?
- How frequently occur?
- What are the consequences?

2.5.1.1 Steps of Risk Assessment process

To start a Risk Assessment process, it is necessary to follow a set of steps that the ICH Q9 guide suggests **(Figure 5)**. The first step is **Risk Identification** that consists of identifying the hazards of the entire manufacturing process, referring to the Risk question or problem description. Essentially, identifying the hazards answers the question, "What can go wrong?". This is a brainstorming activity with the team. This activity should generate many known and potential failure modes. The goal is to make this list as exhaustive as possible. Since identifying all potential failures can be an enormous task when considering an entire manufacturing process, the process map generated during the initiation phase of the risk management process is a valuable tool to generate the logical breaks that organize and focus the brainstorming [34][39].

The next step in the Risk Assessment is **Risk Analysis**. It is the qualitative or quantitative process of linking the likelihood of occurrence and severity of harms. The qualitative or quantitative estimation of severity or the consequence, and the likelihood and the ability to detect the failure, are determined during the risk analysis [34].

The final step in the Risk Assessment is the **Risk Evaluation**. In this stage are compared the identified and analysed Risk against given risk criteria. At this point, the risk assessment phase ends [34].

Figure 5. Risk Assessment process.

The risk management process would continue through the steps of risk control, risk review and communication **(Figure 4)**.

2.5.2 Risk Control

Risk control includes decision making to reduce and/or accept risks. The purpose of risk control is to reduce the risk to an acceptable level. The amount of effort used for risk control should be proportional to the significance of the risk. Decision makers might use different processes, including benefit-cost analysis, for understanding the optimal level of risk control [34].

Risk control might focus on the following questions:

- Is the risk above an acceptable level?
- What can be done to reduce or eliminate risks?
- What is the appropriate balance among benefits, risks and resources?
- Are new risks introduced as a result of the identified risks being controlled?

Risk reduction focuses on processes for mitigation or avoidance of quality risk when it exceeds a specified (acceptable) level **(Figure 4)**. Risk reduction might include actions taken to mitigate the severity and probability of damage. Processes that improve the detectability of hazard and quality risks might also be used as part of a risk control approach. The implementation of risk reduction measures can introduce new risks into the system or increase the significance of other existing risks. Hence, it might be appropriate to revisit the risk assessment to identify and evaluate any possible change in risk after implementing a risk reduction process [34].

Risk acceptance is a decision to accept risk (decided case by case assessment). Risk acceptance can be a formal decision to accept the minor risk or it can be a passive decision in which minor risks are not specified. For some types of harms, even the best quality risk management practices might not eliminate risk .In these circumstances, it might be necessary an appropriate quality risk management support and that quality risk is reduced to a specified (acceptable) level [34].

In some industries, are widely used the Hierarchy of Controls **(Figure 6)** to minimize or eliminate exposure to hazards. The hazard controls in the hierarchy are, in order of decreasing effectiveness:

- Elimination
- Substitution
- Engineering Controls
- Administrative Controls
- Personal protective equipment [40].

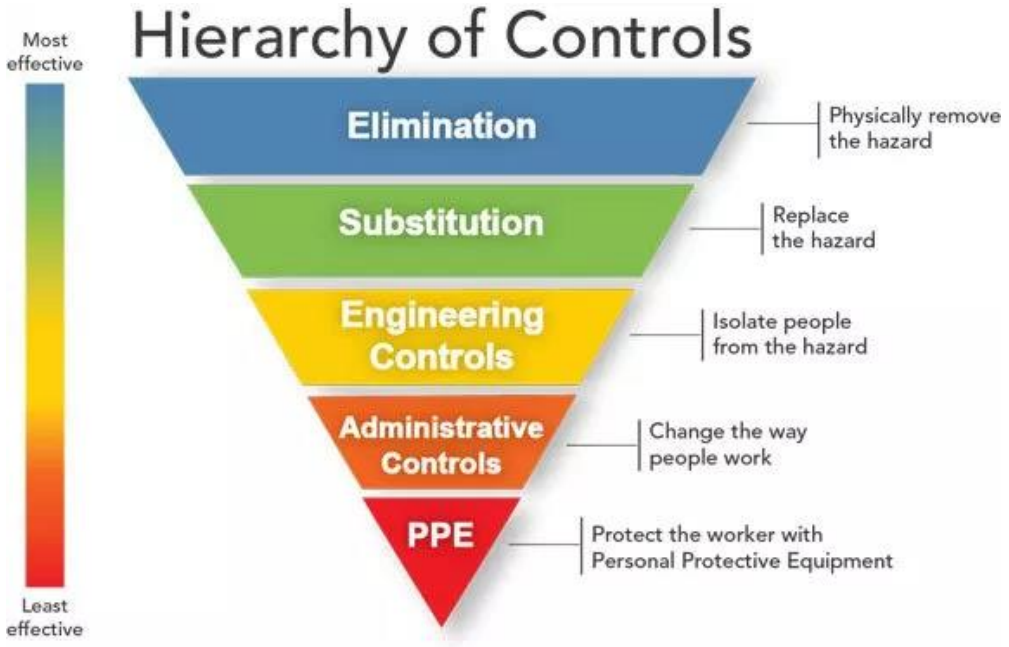

Figure 6. Hierarchy of Controls (40).

The idea behind this hierarchy is that the control methods at the top of graphic are potentially more effective and protective than those at the bottom. Following this hierarchy normally leads to the implementation of inherently safer systems [41].

2.5.3 Risk Communication

Risk communication is the sharing of information about risk and risk management between the decision makers and others. Parties can communicate at any stage of the risk management process **(Figure 4)**. The output/result of the quality risk management process should be appropriately communicated and documented **(Figure 4)**. Communications might include those among interested parties; e.g., regulators and industry, industry and the patient, within a company, industry or regulatory authority, etc. The included information might relate to the existence, nature, form, probability, severity, acceptability, control, treatment, detectability or other aspects of risks to quality. Communication need not be carried out for each and every risk acceptance. Between the industry and regulatory authorities, communication concerning quality risk management decisions might be affected through existing channels as specified in regulations and guidance's [34].

CHAPTER 3 – Materials and Methods

3.1 Data and data processing

In Risk Assessment, the data and their mode of treatment can influence the success of this approach. Due this, in this scientific project, the data collection was performed following two different approaches. To obtain information about the levels of EI present in the API and Excipients, used in the formulation of the drug product, a formulary was sent to the respective suppliers. In absence of a response it was decided assuming the worst-case scenario, that the presence of EI in the component was in the maximum concentration allowed for option 1. This scenario was derived for the remaining calculation options. In the case of the supplier does not perform the EI level tests for the required elements and considering the route of administration to be used, it was decided that the highest value of an element that the supplier has quantified was assumed.

A complete risk analysis includes the identification of all materials that directly contact with drug product, including throughout the manufacture. This material can contribute significantly to the presence of EI in final drug product, in order to obtain the possible contribution, it was performed a bibliographic literature review, in absence of information from the supplier. It is important to note that in many of these extractable studies the conditions under which the tests are carried out more aggressive than the properties of the product. Thus, these tests can overestimate the presence of this elements.

The lack of data and the worst-case scenario negatively influence the outcome of the Risk Assessment, since the results obtained can lead to this analysis for three scenarios. Therefore, the result of the Risk Assessment can be found in the green zone, an area that, even assuming the worst scenario, does not require control actions to be implemented. The results that are in the orange zone, are considered limiting because they are close to the zone that requires control actions. In the red zone are the results of Risk Assessment that exceed the maximum values of control. In this zone control actions are necessary, resorting to the principles described in ICH Q9.

3.2 Methodology for obtaining the PDE's for the ophthalmic route

The Permitted Daily Exposure (PDE) gives the maximum permitted quantity of each element that may be contained in the maximum daily intake of a drug product. In this guidance, the PDE's are considered the to be protective of public health for all patients populations [(2)].

The ICH Q3D, only establishes the PDE's for the Oral, Parenteral and Inhalation routes of administration, however the objective of this scientific work is to apply this guide to ophthalmic products. Thus, it is necessary to obtain the PDE's of each elemental impurity for the ocular route of administration and to do this it is necessary to calculate the ophthalmologic PDE. To perform this approach is necessary to apply the **Equation 1**, were the NO(A)EL means Non-Observed-Effect Level and F1, F2, F3, F4 and F5 represents the modifying factors.

$$PDE = \frac{NO(A)EL \times Mass\ Adusjtment}{F1 \times F2 \times F3 \times F4 \times F5}$$ **(Eq. 1)**

F1 – A factor to account for extrapolation between species.
F2 – A factor 10 to account for variability between individuals.
F3 – A variable factor to account for toxicity studies of short-term exposure.
F4 – A factor that may be applied in cases of severe toxicity.
F5 – A variable factor that may be applied if the NO(A)EL was not established.

Normally, the PDE is preferably derived from a NO(A)EL. If no NO(A)EL is obtained, the LO(A)EL, Lowest-Observed-Effect Level, may be used. Due the lack of information on bioavailability, it was not possible to conclude this procedure and the suggestion of this guidance, derivation of the PDE, was adopted [(2)].

Given the complexity of the eye as an organ, demonstration of appropriate acceptance limits for elemental impurities derived from the ophthalmic route of administration is required. Thus, a specific assessment for elemental impurities exposure and derived risks was performed, with an aim to establish acceptance limits for ophthalmologic products. During this assessment the factors considered were the physiology and the performance of the tear-flow drainage system, the effect of excipients and bioavailability of the drug product on ocular and the ocular toxicological profile of the elemental impurities of interest.

According to the ocular toxicological profile of the elements it is possible indicate that, unless the eye is exposed to excessive levels of particular elements, no local effects or visual impairments are expected. Given that, the major part of the applied dose is absorbed systemically through a non-productive absorption to the conjunctiva of the eye via the nasal mucosa by spillage from the conjunctival sac or loss through the puncta to the lacrimal drainage system. Therefore, it was decided that the Permitted Daily Exposures, for ocular route, in all of the three products included in this scientific work, assume the values provided in ICH Q3D for parenteral route. Due this, no local effects are expected at exposure levels below the parenteral PDE values, thus it was not necessary apply modification factors in parental PDE's [(42)(43)].

In view of the facts above mentioned, for the proposes of the Risk Assessment, the values of the Predicted Daily Exposure of each elemental impurity included on this evaluation are depicted in **Table 2**.

Table 2. Parental Permitted Daily Exposures for Elemental Impurities. Data revived from (2)**.**

Element	Class	Parental Concentration (µg/g)
Cd	1	0.2
Pb	1	0.5
As	1	1.5
Hg	1	0.3
Co	2A	0.5
V	2A	1
Ni	2A	2
Tl	2B	0.8
Au	2B	10
Pd	2B	1
Ir	2B	1
Os	2B	1
Rh	2B	1
Ru	2B	1
Se	2B	8
Ag	2B	1
Pt	2B	1
Li	2B	25
Sb	3	9
Ba	3	70
Mo	3	150
Cu	3	30
Sn	3	60
Cr	3	110

3.3 Calculation the Concentration Limit (CL) to control the levels of EI in the Active Substance and Excipients that composes the drug product.

To control the levels of EI originating from this drug components the ICH Q3D suggest the conversion of the PDE in a CL. The ICH Q3D suggests some acceptable approaches to establishing concentrations of EI in drug product or components that would assure that the drug product does not exceed the PDEs [(2)]. The applicant may select any of these options and assure that the drug product does not exceed the PDE's. In the case of this scientific project, we are test all options except the option 3 because it requires analysis on final drug product and it is not possible to realise.

3.3.1. Option 1:

This option is intended to be used in products with a daily intake (amount) of drug product of 10 g or less. This approach assumes that the elemental impurities, identified during the Risk Assessment, are present in all components of the drug product and which must be tested by assuming a daily intake of 10 g, but in the case of the eye drops solution it was considered that this scenario was greatly overestimated [(2).] Thus, a bibliographic search in Infarmed

database was performed to identify which is the most common type of bottles used in packaging of eye drops solution.

After analysing the results obtained **(Appendix 1)**, it is possible to conclude that the most common types of bottle used in eye drops solution are 5 ml (37%), 10 ml (25%) and 3 ml (8%). According this, it was decided test this option assuming a daily intake of drug product of the 5, 10 and 3 g. Although daily intakes were readjusted, these scenarios are overestimated too, because it is assumed that a bottle of drug product is administrated per each day and according to the posology of an eye drops solution only a few drops are applied.

With this option, the applicant can determine the maximum concentration in µg for each element, assuming these three daily intakes of drug product, using the **Equation 2**.

$$Concentration_{Limit}\ (\mu g/g) = \frac{PDE\ (\mu g/day)}{daily\ amount\ of\ drug\ product\ (g)}$$ ***(Eq. 2)***

The CL is the maximum level of each EI in the drug product. To obtain acceptance by this option, the concentration obtained in drug product can't be equal or more than the CL.

3.3.2. Option 2A:

This option is intended to be applied in a drug product with a specific daily intake. It is very similar to option 1, but in this case the daily intake of drug product is substituted by the MDD of drug product. This option allows, like the option 1, to obtain the CL for each EI, using the **Equation 3**. If all components in a drug product do not exceed the option 2A concentration for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product [(2)].

$$Concentration_{Limit}(\mu g/g) = \frac{PDE\ (\mu g/day)}{maximum\ daily\ dose\ (MDD)\ (g/day)}$$ (**Eq. 3)**

3.3.3. Option 2B:

The applicant can apply this option to obtain the permitted concentration limit of elements in individual components of a drug product with a specific daily intake [(2)].

This option requires the determination of the maximum daily dose for each component and each element. To do this, we should multiply the predicted elemental impurities concentration value for the correspondent component daily dose (obtained by multiplying drug product MDD for the weight of the corresponding excipient) using the **Equation 4** [(2)].

$$PDE\ (\mu g/day) \geq \sum C_K .\ M_k$$ **(Eq. 4)**

K – Index for each of N components in the drug product
C_k – Permitted concentration of the elemental impurity in component K (µg/g)
M_k – Mass of component K in the maximum daily intake of the drug product (g)

3.3.4. Option 3:

This option is intended to be applied in finished product analysis. In this approach the concentration of each element may be measured in the final drug product. It is necessary to determine the maximum permitted concentration of elemental impurity and to do this the option 1 may be used with the maximum total dose of drug product.

3.4. Risk Assessment Process

Risk Assessment is a simple methodology to obtain an overview of the process and allows to make a plant to minimise the hazards of the manufacturing process and the damage associated [(44)]. In the case of this scientific project, the methodology defined will allow obtaining information about the max level of each EI in the drug product and, consequently, if control measures are necessary.

For the construction of a methodology that was reproducible and applied to other pharmaceutical forms, it was decided to follow the general considerations of ICH Q3D. These considerations are based on three steps, which in many cases can be carried out simultaneously [(2)].

1. Identify the known and potential sources of EI.
2. Evaluate the presence of each EI in all sources of EI and compare with the PDE.
3. Summarize the Risk Assessment [(2)].

The proposed methodology **(Appendix 2)** was constructed with the aim of simplifying the approach of ICH Q3D and was tested in three eye drops solutions. This methodology can be applied to any pharmaceutical form, as it has been attempted to be the least specific possible. This tool was created considering the manufacturing process involved, being possible to be readjusted whenever justified. Thus, our methodology is based on seven steps that present a set of decisions that influence the result of the Risk Assessment. **Figure 7** shows, in a simplified way, the methodology implemented to carry out this Risk Assessment and each step of it will be discussed further in this chapter.

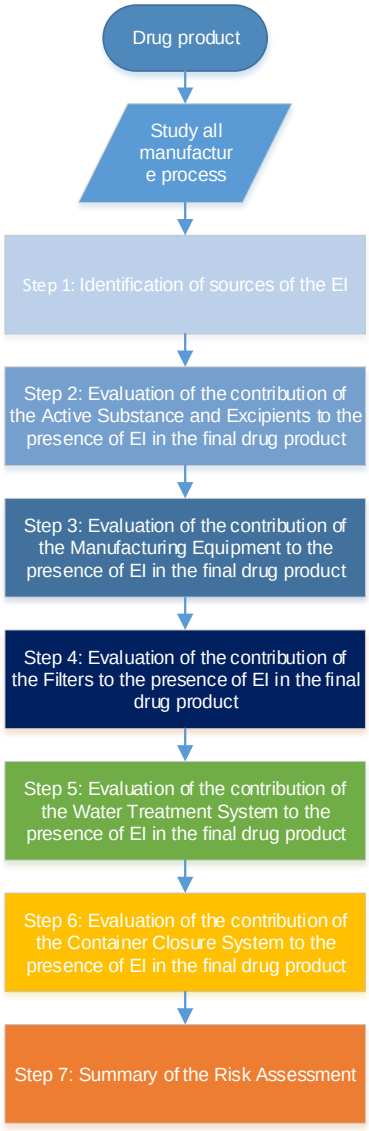

Figure 7. Simplifying methodology to implement the ICH Q3D.

3.4.1. **Step 1:** Identification of sources of the EI:

Identification the sources of elemental impurities is an extremely important step, as this will depend the success of the Risk Assessment. All sources should be exhaustively identified, specially all materials that directly contact with the drug product during the manufacture train and the origin of the Active Substance and Excipients used in the manufacture. These materials and components of drug products can have a greater contribution to the presence of EI in final drug product through any individual or any combination of these sources. This is schematically shown in a fishbone diagram **(Figure 8)**.

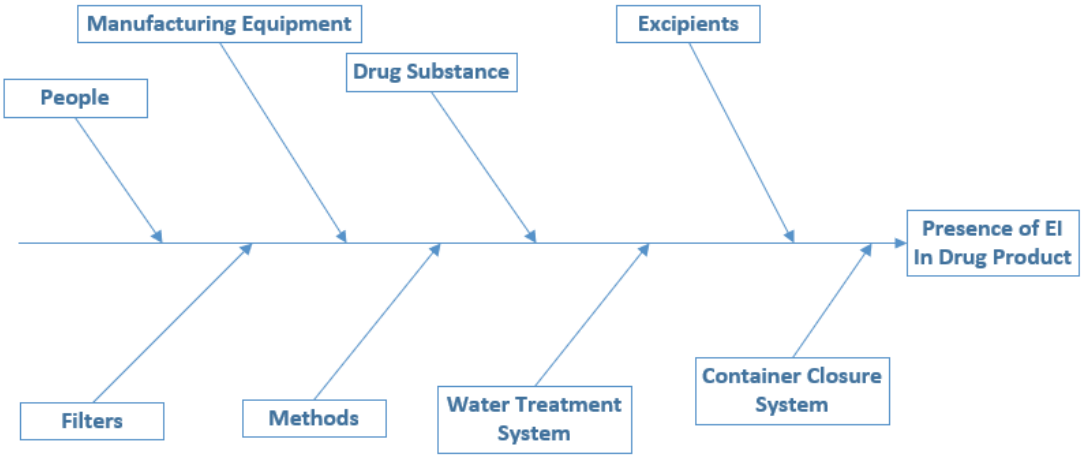

Figure 8. Fishbone diagram showing risk assessment of inclusion of the EI in production of a drug product and potential sources and the overall contribution of the EI to the drug product (adapted by ICH Q3D guideline (2)).

All potential sources of contamination of identified elemental impurities should be included in the Risk Assessment to obtain a perspective of the contribution of each of them in the presence of impurities in the final product. This fishbone diagram was updated from the original diagram, since the methods and people were not included, and these can also contribute a lot to the appearance of impurities.

3.4.2. **Step 2:** Evaluation of the contribution of the Active Substance and Excipients to the presence of EI in the final dur product:

As previously mentioned in this scientific project, the outcome of a risk assessment depends on the data collected. Thus, for the evaluation of the contribution of the Active Substance and the Excipients the respective suppliers were contacted. When the suppliers provide the analytical results regarding the levels of EI in their products these results are used to estimate the presence of EI. On the other hand, when the manufacturer does not provide the analytical results regarding the levels of EI, the worst-case scenario was assumed, the permitted concentration of EI for each element included in this Risk Assessment **(Table 3)**.

The elements included in the Risk Assessment depends mainly of two factors, if they are intentionally added during their manufacture or if any element is leachable from any material that contacts with the Active Substance and Excipient. When no available information, was assumed that the EI are not intentionally added and the Risk Assessment are performed following the recommendations for elements to be considered in the Risk Assessment for Parental route of administration.

After obtaining all elements to be considered in the Risk Assessment, and their respective concentrations, it is necessary to compare the sum of each EI contribution with the CL obtained by Eq. 2, 3 and 4. These equations belong, respectively, to the calculation options 1, 2A and 2B. In this scientific work, these options were tested and if the contribution of each EI does not be equal or more than the CL, the applicant can validate this option and proceed with the Risk Assessment.

Table 3. Permitted Concentration of EI, adapted from (2).

Element	Class	Parental Concentration (µg/g)
Cd	1	0.2
Pb	1	0.5
As	1	1.5
Hg	1	0.3
Co	2A	0.5
V	2A	1
Ni	2A	2
Tl	2B	0.8
Au	2B	10
Pd	2B	1
Ir	2B	1
Os	2B	1
Rh	2B	1
Ru	2B	1
Se	2B	8
Ag	2B	1
Pt	2B	1
Li	2B	25
Sb	3	9
Ba	3	70
Mo	3	150
Cu	3	30
Sn	3	60
Cr	3	110

3.4.3. **Step 3:** Evaluation of the contribution of the Manufacturing Equipment to the presence of EI in the final dur product:

To obtain the contribution of the manufacture equipment it is extremely important identify all equipment involved in production of the drug product. To select the equipment which offers more risk, the equipment that directly contacts with the drug product was identified.

The composition of each manufacturing equipment was obtained after contact with the respective manufacturer of each equipment identified but they only provide analytical the quality certificates. In our case, the Risk Assessment is only performed for the stainless steel. To obtain the elements can be present in this material and their levels are performed a bibliographic search, and in **Figure 9** are depicted the process used to identify the EI present in manufacturing equipment.

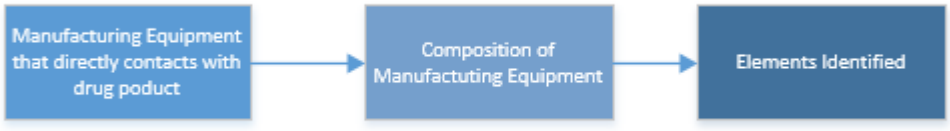

Figure 9. Methodology to identify the elements included in the manufacturing.

To obtain this contribution was assumed a conservative approach, it was assumed 0.5 g/metal leaches from each produced batch to the finished drug product, obtaining an extreme scenario. If the contribution of each EI is less than the PDE, they can be excluded of the Risk Assessment.

3.4.4. **Step 4:** Evaluation of the contribution of the filters to the presence of EI in the final drug product:

Filters are wildly used in many pharmaceutical process. In this particular case, there are three types of filters, but only the Polyvinyl Difluoride (PVDF) and Polypropylene (PP) contact with the drug products. After contact with the manufacturers they do not provide any results regarding to EI levels in filters. To obtain this information it was necessary to perform a literature, and the values obtained represent an overestimated scenario, as they result from extraction tests performed under more aggressive conditions than the product offers.

The contribution of the filters to the presence of EI in final drug product is evaluated by comparing the sum of the contribution of household elemental impurity in each product. This sum is subsequently compared to parenteral PDE.

3.4.5. **Step 5:** Evaluation of the contribution of the Water Treatment System to the presence of EI in the final drug product:

Water is a very common element in most pharmaceutical preparations and easily contaminated. In the case of eye drops solutions, this element is the one that presents the highest percentage in its constitution and it is therefore essential to guarantee its quality. Therefore, it is essential that the pharmaceutical industries have a water treatment system. This system must be properly investigated and all equipment and materials that contact the water throughout the process must be identified to obtain sources of contamination.

After this process, it was decided to contact the suppliers of each identified material, but there was no data about the presence of elemental impurities that could migrate to the drug. It was then necessary to conduct a research to obtain the extraction tests of these materials. The obtained concentrations represent a highly maximized scenario, however, it allows to obtain a maximized perspective of the system.

3.4.6. **Step 6:** Evaluation of the contribution of the Container Closure System to the presence of EI in the final drug product:

The packaging materials may be one of the major sources of EI in the final drug product, given the residence time of the product. In this particular case, the eye drops solution contact directly with Low Density Polyethylene (LDPE) since bottles are made by this polymer. To obtain the concentration of each elements that can leach into the drug product it is extremely

important to obtain the extraction test. The supplier provided the results of the USP Chapter <661> tests, which, although not including the concentration of all the elements, allows a more realistic view of the elements under test. After that, the concentrations were obtained, were compared to the PDEs of the route of administration.

3.4.7. **Step 7:** Summary of the Risk Assessment:

For summary of the Risk Assessment:

- The predicted max contribution for each EI is the highest value observed in all components included in the Risk Assessment.
- The predicted max contribution for each EI is the sum of the contribution of each component included in the Risk Assessment.
- The predicted max contribution for each EI is the sum of each component of the Risk Assessment which hasn't considered **negligible**.

These scenarios were tested using a model table **(Table 4)**, which gives a perspective of the total contribution of each elemental impurity in the final product. Thus, if the total contribution exceeds 30% of the parenteral PDE, it will be necessary to adopt control measures flowing the principles of the Risk Analysis in ICH Q9.

Table 4. Model table that allows to obtain the Summary of the Risk Assessment.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients	Manufacturing Equipment	Filters	Water Treatment System	Container Closure System			
Cd	1								
Pb	1								
As	1								
Hg	1								
Co	2A								
V	2A								
Ni	2A								
⋮	⋮								

CHAPTER 4 – Results and Discussion of Results

This chapter aims is to demonstrate the results obtained by applying the methodology described in chapter **3.4 Risk Assessment process**. This methodology was applied to 3 ophthalmologic products and will be presented in detail in this chapter.

4.1. Drug product 1

4.1.1. Drug product presentation

Drug product 1 is to be administrated topically (ophthalmologic) in patients to reduce intraocular pressure in patients with glaucoma or ocular hypertension. It is formulated with one active substance a selective alpha2-adrenergic receptor agonist [45].

Drug product 1 is presented as eye drops solution being available in packs of 1 dropper bottle of 5 or 10 ml, or 3 bottles of 5 ml. The bottles are made of low density polyethylene (LDPE) and are equipped with LDPE dropper dispenser. To seal the bottles uses a high-density polyethylene (HDPE) caps. The eye drop solution is in direct contact with LDPE bottle and occasionally with the LDPE drop dispenser.

The usual dose of this eye drop solution is one drop in the affected eye(s) twice daily approximately every 12 hours. To be effective, drug product 1 should be administered every day.

In Risk Assessment is essential to determine the Drug Product Maximum Daily Dose (MDD) and to perform this it is necessary to obtain the volume of a single drop of drug product. The drop size of an eye drop solution delivered from plastic dropper bottles depends by three major factors: the design and characteristics of the dropper tip and bottle, the physiochemical properties of the solution to be dispensed, and the patient´s manner of handling the dripper bottle. So, according the literature, the volume of an eye drop may vary from 25 to 70µl (in average 40µl) [46][47][48]. In the case of Laboratório Edol - Produtos Farmacêuticos, S.A. the drop size was determined according to the methodology described in chapter 1151 of USP 37. The Maximum Daily Dose of drug product 1 was determining by **Equation 5** and take in consideration that each drop of drug product 1 has a medium volume of 34.13 µl and considering that are applied 4 drops per day (according to the SPC the maximum application is one drop every 12 hours in each eye).

$$MDD = drop\ size \times dose\ frequency \quad \textbf{(Eq.5)}$$

Assuming the previously mentioned scenario, was obtained an MDD of 0.13 g. **Table 5** shows the quantitative/qualitative composition of drug product 1 and the MDD of each component of drug product.

Table 5. Composition of drug product 1.

Ingredients	Quantity mg/ml	Quantity %	Amount per MDD (g)	Function
Excipient 1	13.86	0.1386	0.0018	Stabilizing agent and humectant agent
Excipient 2	68.32	0.6832	0.0089	Tonicity agent
Excipient 3	4.65	0.0465	0.0006	Buffering system
Excipient 4	0.48	0.0048	0.00006	Buffering system
Excipient 5	0.01	0.0001	0.000001	Preservative
API 1	2.0	0.02	0.00026	API
Highly purified water	Enough for 130L	-	-	Vehicle
May contain hydrochloric acid 1M or sodium hydroxide 1M	As need to pH 6.5 – 7.6	-	-	Adjustment agent

4.1.1.1. Methodology used to perform the Risk Assessment

The methodology implemented to carry out the Risk Assessment to drug product 1 is shown in **(Appendix 2)**. In this methodology the blue arrows represent the chosen options to make the evaluation of the contribution of each EI. There is still, in two stages of this methodology, orange squares that allow to identify the places where both paths have been chosen. There are also steps in this flowchart, which are marked with "*" in red. This symbol allows to identify the critical places of this methodology, that is, places where the decisions taken can lead to different results of the Risk Assessment. It is important to mention that in the last stage of the Risk Assessment, Summary of the Risk Assessment, if any elementary impurity exceeds 30% of the Parenteral PDE, control actions should be taken, using risk analysis and the principles described in ICH Q8 and Q9, however, these actions are already out of the goal of this scientific project. This methodology will be implemented in detail in the following sub-chapters.

4.1.2. Information about components of the drug product to be included in the Risk Assessment

4.1.2.1. API 1

API 1 is an active substance used in formulation of API 1. This drug substance is provided by Farmark, S.A.

The approved and qualified supplier do not provide any analytical results regarding elemental impurities levels in API 1. Thus, to perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.1.2.2. Excipient 1

Excipient 1 is manufactured synthetically and provided by JOSE MANUEL GOMES SANTOS, LDA. The manufacturer of this excipient has provided a declaration about the origin of this product but has not yet provided the analytical results regarding the elemental impurities in this raw material.
To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.1.2.3. Excipient 2

Excipient 2 is synthetically manufactured material, provided by Merck Millipore. (Germany). Manufactured provided a basic dossier (in line with Module 3 CTD Format – Quality) and an Operational Excellence Dossier, concerning Excipient 2suitable for use as excipient EMPROVE® exp Ph.Eur., BP, USP.

Operational Excellence dossier provides information regarding ICH Q3D on elemental impurities in Excipient 2. Such document, presents information on intentionally added elemental impurities during the manufacturing process and an elemental impurity scree, to provide analytical data regarding potentially present Class 1,2 and 3 elements. The manufacturer states “No elements listed in class 1-3 according to the ICH Q3D are used in the manufacturing steps outlined in the manufacturing procedure”.

The manufacture has provided the analytical results of potential present of elemental impurities. The results were obtained by ICP-MS in three commercial batches, as

recommended in ICH Q3D. In **Table 6** only the results concerning class 1, 2A and some of class 3 (Li, SB and Cu) are under assessment, analytical results for class 2B will not be present.

Table 6. Analytical data of elemental impurities in excipient 2, provided by Novo Nordisk Pharmatech.

Risk Assessment summary				
Element	**Class**	**Intentionally added?**	**Concentration (µg/g)**	**Test Method**
Cadmium (Cd)	1	No	≤ 0.06	ICP-MS
Lead (Pb)	1	No	≤ 0.15	
Arsenic (As)	1	No	≤ 0.45	
Mercury (Hg)	1	No	≤ 0.09	
Cobalt (Co)	2A	No	≤ 0.15	
Vanadium (V)	2A	No	≤ 0.3	
Nickel (Ni)	2A	No	≤0.6	
Lithium (Li)	3	No	≤ 7.5	
Antimony (Sb)	3	No	≤ 2.7	
Copper (Cu)	3	No	≤ 9	

4.1.2.4. Excipient 3

Excipient 3 is a manufactured synthetically and provided by JOSE MANUEL GOMES SANTOS, LDA.

Manufacturer has not yet provided analytical results regarding elemental impurities level in Excipient 3.

To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.1.2.5. Excipient 4

Excipient 4 is a synthetically manufactured material, provided by JOSE MANUEL GOMES SANTOS, LDA. Manufacturer has provided a statement of concerning Excipient 4 (USP, BP, JP and Ph.Eur.) pharma grade.

Manufacturer has not provided any analytical results regarding elemental impurities level on this excipient. To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.1.2.6. Excipient 5

Excipient 5 is a preservative used to prevent decomposition by microbial growth or by undesirable chemical changes. This raw material is manufactured by chemical synthesis product. Manufacturer has provided a basic dossier demonstrating that this product can be used as an excipient.

The manufacturer had provided additionally the analytical results regarding the potentially present of elemental impurities, take in consideration the principals described in ICH Q3D and USP Chapter <232>. In **Table 7** only the results concerning class 1, 2A and some of class 3 (Li, SB and Cu) are under assessment, analytical results for class 2B will not be present.

This excipient is manufactured in accordance with the cGMP Guideline ICH Q7 for Active Pharmaceutical Ingredients and it is analysed according to the current European Pharmacopoeia (Ph.Eur.) and United States Pharmacopoeia (USP/NF).

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Table 7. Analytical data of elemental impurities in Excipient 5, provided by Merck Millipore.

Risk Assessment summary				
Element	**Class**	**Intentionally added?**	**Concentration (µg/g)**	**Test Method**
Cadmium (Cd)	1	No	≤ 0.02	ICP-MS
Lead (Pb)	1	No	≤ 0.04	
Arsenic (inorganic)(As)	1	No	≤ 0.03	
Mercury (inorganic) (Hg)	1	No	≤ 0.4	
Cobalt (Co)	2A	No	≤ 0.02	
Vanadium (V)	2A	No	≤ 0.20	
Nickel (Ni)	2A	No	0.10	
Lithium (Li)	3	No	≤ 0.05	
Antimony (Sb)	3	No	≤ 0.03	
Copper (Cu)	3	No	≤ 0.08	

4.1.2.7. Highly Purified Water

Highly purified water is a major component used in formulation of drug product 1. This excipient is manufactured in EDOL and is manufactured in a GMP certified manufacturing site.

The highly purified water is obtained through reverse osmosis from drinking water complying the DL 306/2007 of August 27th, with changes introduced by DL 152/2017 of December 7, and it include controls among others, the level of some elemental impurities, like Arsenic (As),

Cadmium (Cd), Lead (Pb), copper (Cu), Chromium (Cr), Mercury (Hg) and Nickel (Ni). The quality of the water obtained through this process is “Highly purified water” and complies with the “Note for guidance on quality of water for pharmaceutical use CPMP/QWP/158/01 revision)” and with the current Ph.Eur. monograph entitled “Highly Purified Water”. The water production system and treatment system will be addressed in section 4.1.8 Risk Assessment to Water treatment system.

Considering the previous facts, the risk associated to this source is not significant and the predicted levels of elemental impurities contribution in drug product are expected to be **negligible**. For this reason, “Highly Purified Water” will not take in to consideration in the remining Risk Assessment.

4.1.3. Sources of Contamination

The ICH Q3D guideline for EI proposes the potential sources of EI during the manufacturing of a drug product. Each of these potential sources may contribute EI to the drug product, individually or through any combination. **Figure 10** depicts the potential sources of EI that may be found in the drug product 1. During the Risk Assessment, the potential contributions from each of these sources will be considered to determine the overall contribution of elemental impurities to the final product.

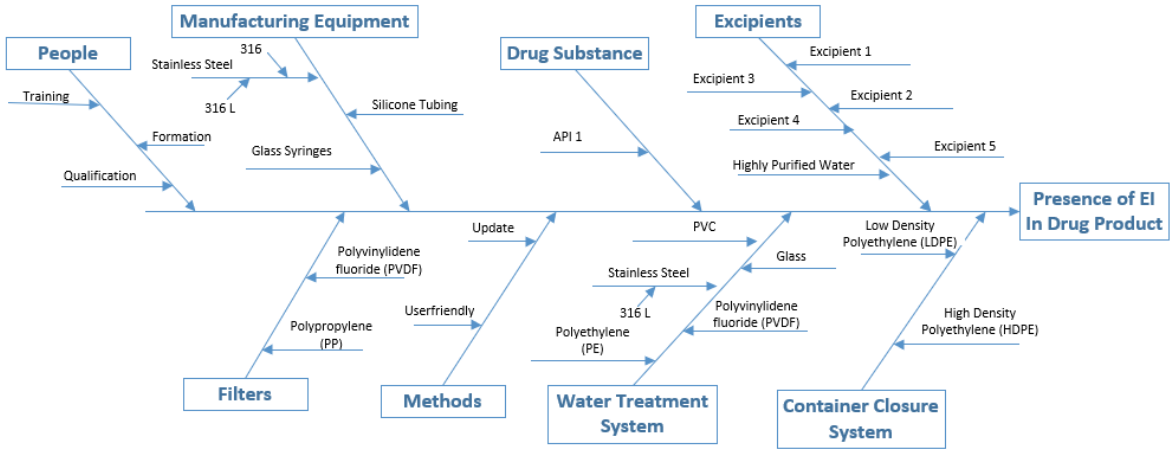

Figure 10. Potential sources of Elemental Impurities in drug product 1.

4.1.4. Elements included in Risk Assessment

Concerning the ICH Q3D recommendations, the elements included in this Risk Assessment are the elements of Class 1, Class 2A and required Class 3 elements, and additionally some other elements intentionally added are included in this Risk Assessment **(Table 8)**.

Table 8. Elements considered in elemental impurities Risk Assessment for drug product 1.

Element	Class	Remarks	Parental PDE (µg/day)	Control threshold 30% PDE (µg/day)
Cd	1	Considered in Active Substance, Excipients, PP, PE and PVC	2	0.6
Pb	1	Considered in Active Substance, Excipients, PP, PVDF, PE, Glass and LDPE	5	1.5
As	1	Considered in Active Substance, Excipients, PP, PE, PVC, Glass and LDPE	15	4.5
Hg	1	Considered in Active Substance, Excipients, PVC and LDPE	3	0.9
Co	2A	Considered in Active Substance, Excipients, PP, PVDF and PVC	5	1.5
V	2A	Considered in Active Substance, Excipients, PP and PVC	10	3
Ni	2A	Considered in Active Substance, Excipients, Stainless steel, PP, PVDF and PVC	20	6
Se	2B	Considered in PP, PE and PVC	80	130
Ag	2B	Considered in PP and PE	10	3
Li	2B	Considered in Active Substance, Excipients, PP and PE	250	75
Sb	3	Considered in Active Substance, Excipients, PP, PE, PVC and LDPE	90	27
Mo	3	Included in composition of Stainless steel, PP and PVC	1500	450
Cu	3	Considered in Active Substance, Excipients, PP, PVDF, PE, PVC and LDPE	300	90
Sn	3	Present in PP, PVDF and PE	600	180
Cr	3	Included in composition of Stainless steel, PP, PVDF, PVC and PE	1100	330

4.1.5. Risk Assessment to Active Substance and Excipients

The analysis of the contribution of the active substance and excipients to the presence of EI in the final product will be carried out following the principles described in ICH Q3D, however we will test the calculation options 1, 2A and 2B. Calculation option 3 will not be tested because it requires analysis of the final product.

4.1.5.1. **Option 1:** Common permitted concentration limits of elements across drug product components for drug products with daily intakes not more than 10 g:

The CL was obtained take in consideration the previous three daily amounts of drug product (3, 5 and 10 g/day). These simulations allow to obtain a perspective of these dosages to estimate the contribution of the active substance and excipients. The results are depicted respectively in **Table 9, 10** and **11.** To have drug product acceptance by this option the predicted level for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 9. Predicted Elemental impurities level in drug product 1 take in consideration the option 1 and assuming one daily amount of drug product of 3 g/day.

Components		API 1	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Excipient 5
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.06	0.2	0.2	0.02
	Pb	0.5	0.5	0.15	0.5	0.5	0.04
	As	1.5	1.5	0.45	1.5	1.5	0.03
	Hg	0.3	0.3	0.09	0.3	0.3	0.4
	Co	0.5	0.5	0.15	0.5	0.5	0.02
	V	1	1	0.3	1	1	0.20
	Ni	2	2	0.6	2	2	0.10
	Li	25	25	7.5	25	25	0.05
	Sb	9	9	2.7	9	9	0.03
	Cu	30	30	9	30	30	0.08

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0.88	2.19	6.48	1.69	2.2	4.5	8.7	107.6	167.8	129.1
Concentration limit (µg/g)	0.67	1.67	5	1	1.67	3.33	6.67	83.3	30	100
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

Table 10. Predicted Elemental impurities level in drug product 1 take in consideration the option 1 and assuming one daily amount of drug product of 5 g/day.

Components		API 1	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Excipient 5
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.06	0.2	0.02	0.2
	Pb	0.5	0.5	0.15	0.5	0.04	0.5
	As	1.5	1.5	0.45	1.5	0.03	1.5
	Hg	0.3	0.3	0.09	0.3	0.4	0.3
	Co	0.5	0.5	0.15	0.5	0.02	0.5
	V	1	1	0.3	1	0.20	1
	Ni	2	2	0.6	2	0.10	2
	Li	25	25	7.5	25	0.05	25
	Sb	9	9	2.7	9	0.03	9
	Cu	30	30	9	30	0.08	30

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0.88	2.19	6.48	1.69	2.17	4.5	8.7	107.55	167.81	129.08
Concentration limit (µg/g)	0.4	1	3	0.6	1	2	4	50	18	60
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

Table 11. Predicted Elemental impurities level in drug product 1 take in consideration the option 1 and assuming one daily amount of drug product of 10 g/day.

Components		API 1	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Excipient 5
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.06	0.2	0.02	0.2
	Pb	0.5	0.5	0.15	0.5	0.04	0.5
	As	1.5	1.5	0.45	1.5	0.03	1.5
	Hg	0.3	0.3	0.09	0.3	0.4	0.3
	Co	0.5	0.5	0.15	0.5	0.02	0.5
	V	1	1	0.3	1	0.20	1
	Ni	2	2	0.6	2	0.10	2
	Li	25	25	7.5	25	0.05	25
	Sb	9	9	2.7	9	0.03	9
	Cu	30	30	9	30	0.08	30

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0.88	2.19	6.48	1.69	2.17	4.5	8.7	107.55	167.81	129.08
Concentration limit (µg/g)	0.2	0.5	1.5	0.3	0.5	1	2	25	9	30
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

It is possible conclude that the adjustment of the daily dose of drug product was not sufficient to obtain the acceptance of this option. This fact can be justified by the worst-case assumed that originated lowers CL when compared with the total EI contribution. It is important to note that the concentrations of each component are also overestimated so that the total value may be higher than the real value. When compared with the PDE, in all cases, the total EI contribution is lower than the PDE.

4.1.5.2. **Option 2A:** Common permitted concentration limits across drug product components for drug products with a specific daily intake:

Maximum Daily Dose (MDD) of drug product 1 is 0.13 g. Acceptance criteria for this option is similar to option 1 thus, the predicted level of elemental impurities for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 12. Predicted Elemental impurities level in drug product 1 take in consideration the option 2A.

Components		API 1	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Excipient 5
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.06	0.2	0.02	0.2
	Pb	0.5	0.5	0.15	0.5	0.04	0.5
	As	1.5	1.5	0.45	1.5	0.03	1.5
	Hg	0.3	0.3	0.09	0.3	0.4	0.3
	Co	0.5	0.5	0.15	0.5	0.02	0.5
	V	1	1	0.3	1	0.20	1
	Ni	2	2	0.6	2	0.10	2
	Li	25	25	7.5	25	0.05	25
	Sb	9	9	2.7	9	0.03	9
	Cu	30	30	9	30	0.08	30

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0.88	2.19	6.48	1.69	2.17	4.5	8.7	107.55	167.81	129.08
Concentration limit (µg/g)	15.4	38.5	115.4	23.1	115.4	76.9	153.8	1923.1	69.2	2307.7
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

After analysis the previous table it is possible conclude that all components in a drug product do not exceed the option 2A CL so, these elements can be used in any proportion in the drug product. When the total EI contribution is compared with the PDE, it is possible verify that the values obtained are a bit far to the parental PDE. For this product, the applicant can be use this option to validate the level of EI in components of drug product.

4.1.5.3. **Option 2B:** Permitted concentration limits in individual components of a product with a specified daily intake:

In option 2B are considered the quantitative composition of the drug product considering the MDD of that specific product.

This option requires the determination of the MDD for each component and each element. To do this, we should multiply the predicted elemental impurities concentration value for the correspondent component daily dose (obtained by multiplying drug product MDD for the weight of the corresponding excipient).

Table 13. Predicted Elemental impurities level take in consideration the option 2B.

Components:		API 1	Excipient 1	Excipient 2	Excipient 3	Excipient 4	Excipient 5
Batch Formula (mg/ml):		2.0	13.86	68.32	4.65	0.48	0.01
MDD (g/day):		0.00026	0.0018	0.0089	0.0006	0.00006	0.000001
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.06	0.2	0.2	0.02
	Pb	0.5	0.5	0.15	0.5	0.5	0.04
	As	1.5	1.5	0.45	1.5	1.5	0.03
	Hg	0.3	0.3	0.09	0.3	0.3	0.4
	Co	0.5	0.5	0.15	0.5	0.5	0.02
	V	1	1	0.3	1	1	0.20
	Ni	2	2	0.6	2	2	0.10
	Li	25	25	7.5	25	25	0.05
	Sb	9	19	2.7	9	19	0.03
	Cu	30	30	9	30	30	0.08
Max daily contribution from excipient (µg/day)	Cd	0,000052	0,00036	0,000534	0,00012	0,00000002	0,000012
	Pb	0,00013	0,0009	0,001335	0,0003	0,00000004	0,00003
	As	0,00039	0,0027	0,004005	0,0009	0,00000003	0,00009
	Hg	0,000078	0,00054	0,000801	0,00018	0,0000004	0,000018
	Co	0,00013	0,0009	0,001335	0,0003	0,00000002	0,00003
	V	0,00026	0,0018	0,00267	0,0006	0,0000002	0,00006
	Ni	0,00052	0,0036	0,00534	0,0012	0,0000001	0,00012
	Li	0,0065	0,045	0,06675	0,015	0,00000005	0,0015
	Sb	0,00234	0,0342	0,02403	0,0054	0,00000003	0,00054
	Cu	0,0078	0,054	0,0801	0,018	0,00000008	0,0018

Total EI contribution (µg/day)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0,001	0,0027	0,008	0,002	0,003	0,005	0,011	0,135	0,067	0,162
Control threshold 30% PDE (µg/day)	0.6	1.5	4.5	0.9	1.5	3	6	75	27	90
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

The option 2B, according to the information provided by **Table 13**, is an approach valid to estimate the contribution of Active Substance and Excipients to presence of EI in final product. The daily contribution of each EI is very low, although the worse scenario has been assumed.

4.2. Drug product 2

4.2.1. Drug product presentation

Drug product 2 is an eye drops solution that has 10 mg/ml of the API 2. This drug belongs to the pharmacotherapeutic group 15.3.2 - Medications used in ocular conditions; mydriatic and cycloplegic; anticholinergics.

Drug product 2 is an anticholinergic that blocks muscarinic acetylcholine receptors, causing paralysis of the circular muscle of the iris and ciliary muscle. This blockage leads to pupil dilation and accommodation paralysis.

This product is intended to be administrated topically for conducting examinations of the fundus of the eye, examinations of refraction and as mydriatic in the treatment of iritis, iridocyclites, choroidites and uveitis.

Drug product 2 is presented in a sterile LDPE bottle equipped with a LDPE dropper dispenser and to seal the bottle is used a HDPE cap. In term of exposure, the solution directly contacts with the LDPE bottle and occasionally with the dropper dispenser. Each bottle contains 5 ml of clear and colourless solution.

The recommended dosage depends on its therapeutic indication, so cycloplegic refraction should be given one drop of drug product 2 (adults), followed by another drop five minutes later. In ophthalmology, this medicinal product should be administered in the same manner as for cycloplegic refraction. In case of Uveitis, a drop should be given 3 to 4 times a day.

In Risk Assessment is essential to determine the Drug Product Maximum Daily Dose (MDD) and to perform this it is necessary to obtain the volume of a single drop of drug product.

The drop size of an ophthalmologic drug product delivered from plastic dropper bottles depends by three factors like the design and characteristics of the dropper tip and bottle, the physiochemical properties of the solution, and the patient´s manner of handling the dripper bottle [46][47][49]. Therefore, to obtain the drop size the drug manufacturer was performed tests according to the USP 37 Chapter 1151. According to the results obtained by each operator we considering that each drop has a medium volume of 31.54 µl, so considering that each drop of drug product 2 has a medium volume of 31.54 µl and the number of drops applied per day are 3 and 4, the MDD for drug product 2, assuming that the patient is performing a two-eye treatment.

The MDD obtained is 0.25 (0.03154x(2x4) and according this, the maximum daily amount for the active substance and the respective excipients used in the formulation of this pharmaceutical form, was calculated and the respective results are in the **Table 14**.

Table 14. Qualitative/quantitative composition of drug product 2.

Ingredients	Quantity mg/ml	Quantity %	Amount per MDD (g)	Function
Excipient 6	7.5	0.075	0.00188	Buffering agent
Excipient 7	4.5	0.045	0.00113	Buffering agent
Excipient 8	0.6	0.006	0.00015	Buffer chelating agent
Excipient 9	0.02	0.0002	0.000005	Buffering agent
Excipient 5	0.2	0.002	0.00005	Preservative
Excipient 2	As needed to 300 – 400 mOsm/Kg	-	-	Osmolarity adjustment agent
API 2	10	0.1	0.0025	API
Highly purified water	Enough for 150 l	-		Vehicle
May contain hydrochloric acid 1M or sodium hydroxide 1M	As need to pH 3.0 – 5.5	-	-	Adjustment agent

4.2.1.1. Methodology used to perform the Risk Assessment

The methodology implemented to carry out the Risk Assessment to drug product 2 eye drops solution is shown in **(Appendix 2)**. In this methodology the blue arrows represent the chosen options to make the evaluation of the contribution of each EI. There is still, in two stages of this methodology, orange squares that allow to identify the places where both paths have been chosen. There are also steps in this flowchart, which are marked with "*" in red. This symbol allows to identify the critical places of this methodology, that is, places where the decisions taken can lead to different results of the Risk Assessment. It is important to mention that in the last stage of the Risk Assessment, Summary of the Risk Assessment, if any elementary impurity exceeds 30% of the Parenteral PDE, control actions should be taken, using risk analysis and the principles described in ICH Q8 and Q9, however, these actions are already out of the goal of this scientific project, so it was decided to finish this methodology as shown in **(Appendix 2)**.This methodology will be implemented in detail in the following sub-chapters.

4.2.2. Information about components of the drug product to be included in the Risk Assessment

4.2.2.1. API 2

API 2 is manufactured by UQUIFA SPAIN, SA, but after contact with this supplier he does not provide any information about the tests performed to evaluate the presence of elemental impurities in their products.

To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.2.2.2. Excipient 5

The information on this excipient is described in section 4.1.2.6 of this scientific project.

4.2.2.3. Excipient 6

Excipient 6 is used in formulation of drug product 2 as a buffering agent. This product is manufactured by PANREAC and after contact with this manufacturer he does not provide any analytical results regarding elemental impurities levels on Excipient 6.

To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.2.2.4. Excipient 7

Excipient 7 is a buffering agent used in the formulation of drug product 2. This product is synthetically and provided by VWR INTERNACIONAL, LDA. Manufactured provided the basic dossier, concerning Excipient 7 suitable for the biopharmaceutical production EMPROVE® bio Ph.Eur, BP, USP.

After contact with this supplier he does not provide any information about the tests performed to evaluate the presence of elemental impurities in their products, so to perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.2.2.5. Excipient 8

Excipient 8 is synthetically manufactured material, provided by Merck Millipore. This excipient is used in the formulation as a Buffer chelating agent in very lower amounts.

After contact with this manufacturer he does not provide any analytical results regarding elemental impurities levels on Excipient 8. So, to perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.2.2.6. Excipient 9

Excipient 9 is synthetically manufactured material, provided by PANREAC and after contact with the manufacturer 3V SIGMA USA, he informs it can be expected traces of heavy metals (as Co, Ni, Mo, Cr), but the metals are technically unavoidable in the manufacturing process and are not intentionally added during the manufacturing process. This manufactured does not provide any analytical results regarding elemental impurities levels on Excipient 9.

To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, Sb and Cu) are the permitted concentration of elemental impurities described in ICH Q3D.

4.2.2.7. Excipient 2

The information on this excipient is described in section 4.1.2.3 of this scientific project.

4.2.2.8. Highly Purified Water

The information on this excipient is described in section 4.1.2.7 of this scientific project.

4.2.3. Sources of Contamination

The ICH Q3D guideline for EI proposes the potential sources of EI during the manufacturing of a drug product. Each of these potential sources may contribute EI to the drug product, individually or through any combination. **Figure 12** depicts the potential sources of EI that may be found in the drug product 2. During the Risk Assessment, the potential contributions from each of these sources will be considered to determine the overall contribution of elemental impurities to the drug product.

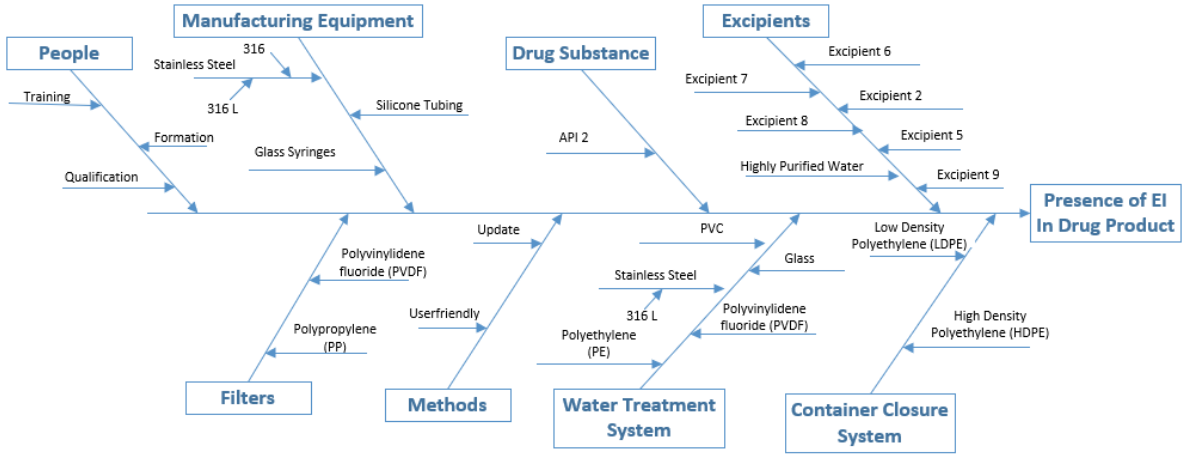

Figure 11. Potential sources of Elemental Impurities in drug product 2.

4.2.4. Elements included in Risk Assessment

ICH Q3D provide recommendations for inclusion of elemental impurities in the risk assessment, due this, the elements considered for this Risk Assessment are based on the recommendation of the ICH Q3D. In this respect, the elements included are these elements of Class 1, Class 2A and required Class 3 elements, and additionally some other elements intentionally added are added in **Table 15**.

Element	Class	Remarks	Parental PDE (µg/day)	Control threshold 30% PDE (µg/day)
Cd	1	Considered in Active Substance, Excipients, PP, PE and PVC	2	0.6
Pb	1	Considered in Active Substance, Excipients, PP, PVDF, PE, Glass and LDPE	5	1.5
As	1	Considered in Active Substance, Excipients, PP, PE, PVC, Glass and LDPE	15	4.5
Hg	1	Considered in Active Substance, Excipients, PVC and LDPE	3	0.9
Co	2A	Considered in Active Substance, Excipients, PP, PVDF and PVC	5	1.5
V	2A	Considered in Active Substance, Excipients, PP and PVC	10	3
Ni	2A	Considered in Active Substance, Excipients, Stainless steel, PP, PVDF and PVC	20	6
Se	2B	Considered in PP, PE and PVC	80	130
Ag	2B	Considered in PP and PE	10	3
Li	2B	Considered in Active Substance, Excipients, PP and PE	250	75
Sb	3	Considered in Active Substance, Excipients, PP, PE, PVC and LDPE	90	27
Mo	3	Included in composition of Stainless steel, PP and PVC	1500	450
Cu	3	Considered in Active Substance, Excipients, PP, PVDF, PE, PVC and LDPE	300	90
Sn	3	Present in PP, PVDF and PE	600	180
Cr	3	Included in composition of Stainless steel, PP, PVDF, PVC and PE	1100	330

4.2.5. Risk Assessment to Active Substance and Excipients

The analysis of the contribution of the active substance and excipients to the presence of EI in the final product will be carried out following the principles described in ICH Q3D, however we will test the calculation options 1, 2A and 2B. Calculation option 3 will not be tested because it requires analysis of the final product.

4.2.5.1. **Option 1:** Common permitted concentration limits of elements across drug product components for drug products with daily intakes not more than 10 g:

Previously, in the in chapter of methodology, the CL was obtained assuming three daily amounts of drug product (3, 5 and 10 g/day). The results of this simulations are depicted respectively in **Tables 16, 17 and 18**. In order to have drug product acceptance by this option the predicted level for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 16. Predicted Elemental impurities level in drug product 2 take in consideration the option 1 and assuming one daily amount of drug product of 3 g/day.

Components		API 2	Excipient 6	Excipient 7	Excipient 8	Excipient 9	Excipient 5	Excipient 2
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.2	0.2	0.2	0.02	0.06
	Pb	0.5	0.5	0.5	0.5	0.5	0.04	0.15
	As	1.5	1.5	1.5	1.5	1.5	0.03	0.45
	Hg	0.3	0.3	0.3	0.3	0.3	0.4	0.09
	Co	0.5	0.5	0.5	0.5	0.5	0.02	0.15
	V	1	1	1	1	1	0.20	0.3
	Ni	2	2	2	2	2	0.10	0.6
	Li	25	25	25	25	25	0.05	7.5
	Sb	9	9	9	9	9	0.03	2.7
	Cu	30	30	30	30	30	0.08	9

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	1.08	2.69	7.98	1.99	2.67	5.5	10.7	132.6	47.73	159.1
Concentration limit (µg/g)	0.67	1.67	5	1	1.67	3.33	6.67	83.3	30	100
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

Table 17. Predicted Elemental impurities level in drug product 2 take in consideration the option 1 and assuming one daily amount of drug product of 5 g/day.

Components		API 2	Excipient 6	Excipient 7	Excipient 8	Excipient 9	Excipient 5	Excipient 2
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.2	0.2	0.2	0.02	0.06
	Pb	0.5	0.5	0.5	0.5	0.5	0.04	0.15
	As	1.5	1.5	1.5	1.5	1.5	0.03	0.45
	Hg	0.3	0.3	0.3	0.3	0.3	0.4	0.09
	Co	0.5	0.5	0.5	0.5	0.5	0.02	0.15
	V	1	1	1	1	1	0.20	0.3
	Ni	2	2	2	2	2	0.10	0.6
	Li	25	25	25	25	25	0.05	7.5
	Sb	9	9	9	9	9	0.03	2.7
	Cu	30	30	30	30	30	0.08	9

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	1.08	2.69	7.98	1.99	2.67	5.5	10.7	132.6	47.73	159.1
Concentration limit (µg/g)	0.4	1	3	0.6	1	2	4	50	18	60
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

Table 18. Predicted Elemental impurities level in drug product 2 take in consideration the option 1 and assuming one daily amount of drug product of 10 g/day.

Components		API 2	Excipient 6	Excipient 7	Excipient 8	Excipient 9	Excipient 5	Excipient 2
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.2	0.2	0.2	0.02	0.06
	Pb	0.5	0.5	0.5	0.5	0.5	0.04	0.15
	As	1.5	1.5	1.5	1.5	1.5	0.03	0.45
	Hg	0.3	0.3	0.3	0.3	0.3	0.4	0.09
	Co	0.5	0.5	0.5	0.5	0.5	0.02	0.15
	V	1	1	1	1	1	0.20	0.3
	Ni	2	2	2	2	2	0.10	0.6
	Li	25	25	25	25	25	0.05	7.5
	Sb	9	9	9	9	9	0.03	2.7
	Cu	30	30	30	30	30	0.08	9

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	1.08	2.69	7.98	1.99	2.67	5.5	10.7	132.6	47.73	159.1
Concentration limit (µg/g)	0.2	0.5	1.5	0.3	0.5	1	2	25	9	30
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	No	No	No	No	No	No	No	No	No	No

As seen in the previous tables, none of the daily intake of drug products tested was approved. This fact can be explained by the assumed conditions, since these are overestimated and beyond that the daily doses are much higher than the reality. Therefore, this option is not the best to obtain the contribution of EI from Active Substance and Excipients.

4.2.5.2. **Option 2A:** Common permitted concentration limits across drug product components for drug products with a specific daily intake:

Option 2A is similar to option 1 since it also considers that all components in the formulation contribute with the same elemental impurities amount to drug product total elemental impurity level. Distinguishing these two options is the fact that Option 2A considers that actual MDD of the drug product. The MDD of drug product 2 is 0.25 g.

Acceptance criteria for this option is similar to option 1 thus, the predicted level of elemental impurities for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 19. Predicted Elemental impurities level in drug product 2 take in consideration the option 2A.

Components		API 2	Excipient 6	Excipient 7	Excipient 8	Excipient 9	Excipient 5	Excipient 2
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.2	0.2	0.2	0.02	0.06
	Pb	0.5	0.5	0.5	0.5	0.5	0.04	0.15
	As	1.5	1.5	1.5	1.5	1.5	0.03	0.45
	Hg	0.3	0.3	0.3	0.3	0.3	0.4	0.09
	Co	0.5	0.5	0.5	0.5	0.5	0.02	0.15
	V	1	1	1	1	1	0.20	0.3
	Ni	2	2	2	2	2	0.10	0.6
	Li	25	25	25	25	25	0.05	7.5
	Sb	9	9	9	9	9	0.03	2.7
	Cu	30	30	30	30	30	0.08	9

Total EI contribution (µg/g)	**Cd**	**Pb**	**As**	**Hg**	**Co**	**V**	**Ni**	**Li**	**Sb**	**Cu**
	1.08	2.69	7.98	1.99	2.67	5.5	10.7	132.55	47.73	159.1
Concentration limit (µg/g)	8	20	60	12	20	40	80	1000	360	1200
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

After analysis the previous table it is possible conclude that all components in a drug product do not exceed the option 2A CL so, these elements can be used in any proportion in the drug product. When the total EI contribution is compared with the PDE, it is possible verify that the values obtained are a bit far to the parental PDE. For this product, the applicant can be use this option to validate the level of EI in components of drug product.

4.2.5.3. **Option 2B:** Permitted concentration limits in individual components of a product with a specified daily intake:

In option 2B are considered the quantitative composition of the drug product considering the Maximum Daily Dose of that specific product. This option requires the determination of the maximum daily dose for each component and each element. To do this, we should multiply the predicted elemental impurities concentration value for the correspondent component daily dose (obtained by multiplying drug product MDD for the weight of the corresponding excipient).

Table 20. Predicted Elemental impurities level of drug product 2 take in consideration the option 2B.

Components:		API 2	Excipient 6	Excipient 7	Excipient 8	Excipient 9	Excipient 5	Excipient 2
Batch Formula (mg/ml):		10	7.5	4.5	0.6	0.02	0.2	0.6
MDD [1] (g/day):		0.0025	0.00188	0.00113	0.00015	0.000005	0.00005	0.00015
Concentrations of EI (µg/g)	Cd	0.2	0.2	0.2	0.2	0.2	0.02	0.2
	Pb	0.5	0.5	0.5	0.5	0.5	0.04	0.5
	As	1.5	1.5	1.5	1.5	1.5	0.03	1.5
	Hg	0.3	0.3	0.3	0.3	0.3	0.4	0.3
	Co	0.5	0.5	0.5	0.5	0.5	0.02	0.5
	V	1	1	1	1	1	0.20	1
	Ni	2	2	2	2	2	0.10	2
	Li	25	25	25	25	25	0.05	25
	Sb	9	9	9	9	9	0.03	9
	Cu	30	30	30	30	30	0.08	30
Max daily contribution from excipient (µg/day)	Cd	0.0005	0.000376	0.000226	0.00003	0.000001	0.000001	0.00003
	Pb	0.00125	0.00094	0.000565	0.000075	0.0000025	0.000002	0.000075
	As	0.00375	0.00282	0.001695	0.000225	0.0000075	0.0000015	0.000225
	Hg	0.00075	0.000564	0.000339	0.000045	0.0000015	0.00002	0.000045
	Co	0.00125	0.00094	0.000565	0.000075	0.0000025	0.000001	0.000075
	V	0.0025	0.00188	0.00113	0.00015	0.000005	0.00001	0.00015
	Ni	0.005	0.00376	0.00226	0.0003	0.00001	0.000005	0.0003
	Li	0.0625	0.047	0.02825	0.00375	0.000125	0.0000025	0.00375
	Sb	0.0225	0.01692	0.01017	0.00135	0.000045	0.0000015	0.00135
	Cu	0.075	0.0564	0.0339	0.0045	0.00015	0.000004	0.0045

Total EI contribution (µg/day)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Cu
	0.001	0.003	0.009	0.002	0.003	0.006	0.012	0.145	0.052	0.174
Control threshold 30% PDE (µg/day)	0.6	1.5	4.5	0.9	1.5	3	6	75	27	90
PDE (µg/day)	2	5	15	3	5	10	20	250	90	300
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

According to the information provided by **Table 20**, the option 2B for API 2 is valid to estimate the daily contribution of EI by Active Substance and Excipients. The total EI contribution obtained is very low when compared with control threshold 30% of parental PDE and much more when compared with the parental PDE.

4.3. Drug Product 3

4.3.1. Drug product presentation

Drug product 3 is a product indicated to use in acute situations like conjunctivitis, blepharitis, dacryocystitis, corneal ulcers, trachoma and keratitis and are formulated in two pharmaceutical forms, in ointments and in eye drops solution. According the scope of this scientific project, the focus is on eye drops solution.

This eye drops solution is formulated with one drug substance (a broad-spectrum antibiotic and marked antimicrobial activity, to be administrated topically (ophthalmologic) in patients. In the **Table 21**, are depicted the quantitative/qualitative composition of drug product 3 [(50)].

Drug product 3 is presented as a colorless, clear and odorless solution in a 10 ml sterile dropper bottle made of LDPE and each bottle contains 5ml of drug product. The bottle is equipped with LDPE dropper dispenser and to seal the dropper uses a HDPE cap. The eye drop solution is in direct contact with LDPE bottle and occasionally with the LDPE drop dispenser.

In Risk Assessment is essential to determine the Drug Product MDD and to perform this it is necessary to obtain the volume of a single drop of drug product.

The drop size of an ophthalmologic drug product delivered from plastic dropper bottles depends by three major factors: the design and characteristics of the dropper tip and bottle, the physiochemical properties of the solution to be dispensed, and the patient´s manner of handling the dripper bottle. So, according the literature, the volume of an eye drop may vary from 25 to 70µl (in average 40µl) [46][47][48]. To obtain the correct drop size the drug manufacturer was realized tests according to USP 37, chapter 1151. This test revealed that the drop size of the product is approximately 32.25 µl. So, to determine the MDD of drug product 3 was considered that each drop has a medium volume of 35.25 µl and the number of drops applied per day are 32 (according to the SPC the maximum application is two drops every two hours, however it is considered that the patient will not apply the pharmaceutical from during the night (8 hours), thus the number of applications per day are 16, for both eyes). The MDD obtained is (0.03525x32) 1.13g.

Table 21. Composition of drug product 3 per bottle with 5 ml of solution.

Ingredients	Quantity mg/ml	Quantity %	Amount per MDD (g)	Function
API 3	8.00	0.80	0.009	API
Excipient 6	7.70	0.77	0.0087	Buffering system
Excipient 10	1.00	0.10	0.001	Buffering system
Excipient 2	3.50	0.35	0.00396	Tonicity agent
Excipient 5	0.20	0,02	0.00023	Preservative
Excipient 11	26.00	2.6	0.029	Stabilizing agent
Highly purified water	As need to 1.00	-	-	Vehicle
May contain hydrochloric acid 10% or sodium hydroxide 40%	As need to pH 6.5 – 7.6	-	-	Adjustment agent

4.3.1.1. Methodology used to perform the Risk Assessment

The methodology implemented to carry out the Risk Assessment to drug product 3 is shown in **(Appendix 2)**. In this methodology the blue arrows represent the chosen options to make the evaluation of the contribution of each EI. There is still, in two stages of this methodology, orange squares that allow to identify the places where both paths have been chosen. There are also steps in this flowchart, which are marked with "*" in red. This symbol allows to identify the critical places of this methodology, that is, places where the decisions taken can lead to different results of the Risk Assessment. It is important to mention that in the last stage of the Risk Assessment, Risk Summing, if any elementary impurity exceeds 30% of the Parenteral PDE, control actions should be taken, using risk analysis and the principles described in ICH Q8 and Q9, however, these actions are already out of the goal of this scientific project, so it

was decided to finish this methodology as shown in **(Appendix 2)**.This methodology will be implemented in detail in the following sub-chapters.

4.3.2. Information about components of the drug product to be included in the Risk Assessment

4.3.2.1. API 3

API 3 is an Active Substance manufactured at Química Sintética, S.A.

The manufacturer has provided the analytical results of potentially present elemental impurities and had evaluate the different potential sources of elemental impurities as required by ICH Q3D. Analytical data was determined through ICP-MS in three commercial batches, as recommended by ICH Q3D. All concentrations obtained are presented in **Table 22**. It is important to refer that the manufacturer only assess class1, 2A and some class 3 elements (Mo and Cr). Therefore, for the other elements included in Class 3 (Li, Sb, Cu) we were assumed the higher concentration obtained in analytical results of manufacturer.

Table 22. Analytical data of elemental impurities in API 3, provided by Química Sintética, S.A.

Risk Assessment summary				
Element	Class	Intentionally added?	Concentration (µg/g)	Test Method
Cadmium (Cd)	1	No	< 0.06	ICP-MS
Lead (Pb)	1	No	< 0.15	
Arsenic (As)	1	No	< 0.15	
Mercury (Hg)	1	No	< 0.9	
Cobalt (Co)	2A	No	< 0.15	
Vanadium (V)	2A	No	< 0.1	
Nickel (Ni)	2A	No	< 2.5	
Lithium (Li)	3	No	-	
Molybdenum (Mo)	3	No	< 0.3	
Antimony (Sb)	3	No	-	
Copper (Cu)	3	No	-	
Chromium (Cr)	3	No	<0.8	

4.3.2.2. Excipient 6

The information on this excipient is described in section 4.2.2.3 of this scientific project.

4.3.2.3. Excipient 10

Excipient 10 is synthetically manufactured, provided by VWR INTERNATIONAL, LDA. Manufacturer has provided the a statement of compliance with ICH Q3D concerning Excipient 10 suitable for use as excipient EMPROVE® exp Ph.Eur,, BP, NF.

Manufacturer has not provided the analytical results regarding elemental impurities levels on Excipient 10. To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, MO, Sb, Cu and Cr) are the permitted concentration of elemental impurities described in ICH Q3D.

4.3.2.4. Excipient 2

The information on this excipient is described in section 4.1.2.3 of this scientific project.

4.3.2.5. Excipient 11

Excipient 11 is a stabilizing agent using in formulation of drug product 3. This raw material is provided by WACKER CHEMIE AG.

Manufacturer has not provided the analytical results regarding elemental impurities levels on Excipient 11. To perform a Risk Evaluation, it was assumed the worst-case, the concentration of the elements of Class 1, 2B and some of class 3 (Li, MO, Sb, Cu and Cr) are the permitted concentration of elemental impurities described in ICH Q3D.

4.3.2.6. Excipient 5

The information on this excipient is described in section 4.1.2.6 of this scientific project.

4.3.2.7. Highly Purified Water

The information on this excipient is described in section 4.1.2.7 of this scientific project.

4.3.3. Sources of Contamination

The ICH Q3D guideline for EI proposes the potential sources of EI during the manufacturing of a drug product. Each of these potential sources may contribute EI to the drug product, individually or through any combination. **Figure 14** depicts the potential sources of EI that may be found in the drug product drug product 3 eye drops solution. During the Risk Assessment, the potential contributions from each of these sources will be considered to determine the overall contribution of elemental impurities to the drug product.

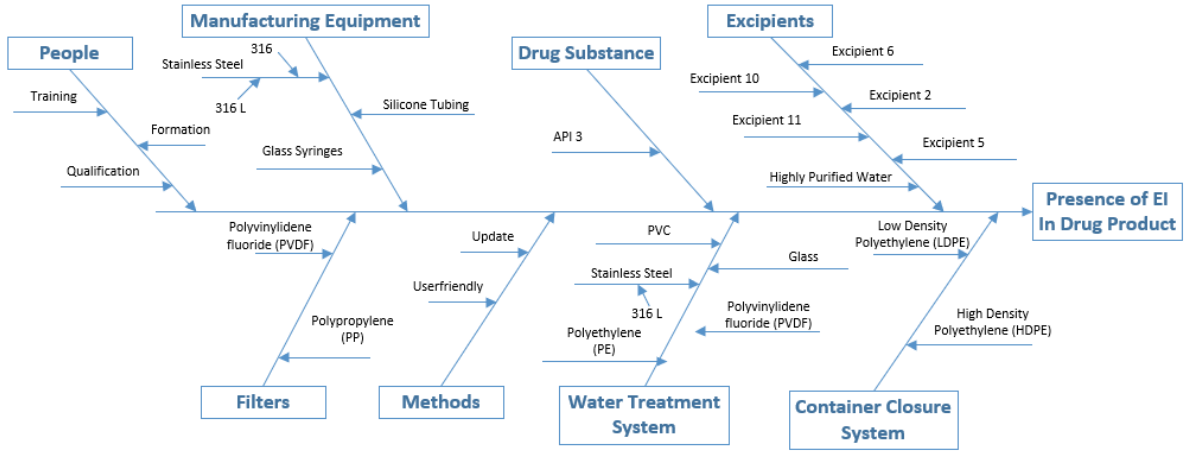

Figure 12. Potential sources of Elemental Impurities in drug product 3.

4.3.4. Elements included in Risk Assessment

ICH Q3D provide recommendations for inclusion of elemental impurities in the risk assessment, due this, the elements considered for this Risk Assessment are based on the recommendation of the ICH Q3D. In this respect, the elements included are these elements of Class 1, Class 2A and required Class 3 elements, and additionally some other elements intentionally added are added in this Risk Assessment in **Table 23**.

Table 23. Elements considered in elemental impurities Risk Assessment for drug product 3.

Element	Class	Remarks	Parental PDE (µg/day)	Control threshold 30% PDE (µg/day)
Cd	1	Considered in Active Substance, Excipients, PP, PE and PVC	2	0.6
Pb	1	Considered in Active Substance, Excipients, PP, PVDF, PE, Glass and LDPE	5	1.5
As	1	Considered in Active Substance, Excipients, PP, PE, PVC, Glass and LDPE	15	4.5
Hg	1	Considered in Active Substance, Excipients, PVC and LDPE	3	0.9
Co	2A	Considered in Active Substance, Excipients, PP, PVDF and PVC	5	1.5
V	2A	Considered in Active Substance, Excipients, PP and PVC	10	3
Ni	2A	Considered in Active Substance, Excipients, Stainless steel, PP, PVDF and PVC	20	6
Se	2B	Considered in PP, PE and PVC	80	130
Ag	2B	Considered in PP and PE	10	3
Li	2B	Considered in Active Substance, Excipients, PP and PE	250	75
Sb	3	Considered in Active Substance, Excipients, PP, PE, PVC and LDPE	90	27
Mo	3	Considered in Active substance and Excipients, in composition of Stainless steel, PP and PVC	1500	450
Cu	3	Considered in Active Substance, Excipients, PP, PVDF, PE, PVC and LDPE	300	90
Sn	3	Present in PP, PVDF and PE	600	180
Cr	3	Considered in Active Substance, Excipients, composition of Stainless steel, PP, PVDF, PVC and PE	1100	330

4.3.5. Risk Assessment to Active Substance and Excipients

The analysis of the contribution of the active substance and excipients to the presence of EI in the final product will be carried out following the principles described in ICH Q3D, however we will test the calculation options 1, 2A and 2B. Calculation option 3 will not be tested because it requires analysis of the final product.

4.3.5.1. **Option 1:** Common permitted concentration limits of elements across drug product components for drug products with daily intakes not more than 10 g:

Option 1 takes into consideration that the MDD of drug product does not exceed 10 g/day. Also, it considers that all the components in the formulation contribute with the same elemental impurities amount to the drug product total concentration.

Like we said previously in the in chapter of methodology, the concentration limit (CL) was obtained assuming three daily amounts of drug product (3, 5 and 10 g/day). The results of this simulations are depicted respectively in **Tables 24, 25** and **26**. In order to have drug product acceptance by this option the predicted level for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 24. Predicted Elemental impurities level in drug product 3 take in consideration the option 1 and assuming one daily amount of drug product of 3 g/day.

Components		API 3	Excipient 6	Excipient 10	Excipient 2	Excipient 5	Excipient 11
Concentrations of EI (µg/g)	Cd	0.06	0.2	0.2	0.06	0.02	0.2
	Pb	0.15	0.5	0.5	0.15	0.04	0.5
	As	0.15	1.5	1.5	0.45	0.03	1.5
	Hg	0.9	0.3	0.3	0.09	0.4	0.3
	Co	0.15	0.5	0.5	0.15	0.02	0.5
	V	0.1	1	1	0.3	0.20	1
	Ni	2.5	2	2	0.6	0.10	2
	Li	2.5	25	25	7.5	0.05	25
	Sb	2.5	9	9	2.7	0.03	9
	Mo	0.3	30	30	9	0.08	30
	Cu	2.5	0.2	0.2	9	0.02	0.2
	Cr	0.8	0.5	0.5	9	0.04	0.5

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Mo	Cu	Cr
	0.74	1.84	5.13	2.29	1.82	3.6	9.2	85.1	32.2	99.4	12.1	11.3
Concentration limit (µg/g)	0.67	1.67	5	1	1.67	3.33	6.67	83.3	30	500	100	366.7
PDE (µg/day)	2	5	15	3	5	10	20	250	90	1500	300	1100
Acceptance	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes

Table 25. Predicted Elemental impurities level in drug product 3 take in consideration the option 1 and assuming one daily amount of drug product of 5 g/day.

Components		API 3	Excipient 6	Excipient 10	Excipient 2	Excipient 5	Excipient 11
Concentrations of EI (µg/g)	Cd	0.06	0.2	0.2	0.06	0.02	0.2
	Pb	0.15	0.5	0.5	0.15	0.04	0.5
	As	0.15	1.5	1.5	0.45	0.03	1.5
	Hg	0.9	0.3	0.3	0.09	0.4	0.3
	Co	0.15	0.5	0.5	0.15	0.02	0.5
	V	0.1	1	1	0.3	0.20	1
	Ni	2.5	2	2	0.6	0.10	2
	Li	2.5	25	25	7.5	0.05	25
	Sb	2.5	9	9	2.7	0.03	9
	Mo	0.3	30	30	9	0.08	30
	Cu	2.5	0.2	0.2	9	0.02	0.2
	Cr	0.8	0.5	0.5	9	0.04	0.5

Total EI contribution (µg/g)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Mo	Cu	Cr
	0.74	1.84	5.13	2.29	1.82	3.6	9.2	85.1	32.2	99.4	12.1	11.3
Concentration limit (µg/g)	0.4	1	3	0.6	1	2	4	50	18	300	60	220
PDE (µg/day)	2	5	15	3	5	10	20	250	90	1500	300	1100
Acceptance	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes

Table 26. Predicted Elemental impurities level in drug product 3 take in consideration the option 1 and assuming one daily amount of drug product of 10 g/day.

Components		API 3	Excipient 6	Excipient 10	Excipient 2	Excipient 5	Excipient 11
Concentrations of EI (µg/g)	Cd	0.06	0.2	0.2	0.06	0.02	0.2
	Pb	0.15	0.5	0.5	0.15	0.04	0.5
	As	0.15	1.5	1.5	0.45	0.03	1.5
	Hg	0.9	0.3	0.3	0.09	0.4	0.3
	Co	0.15	0.5	0.5	0.15	0.02	0.5
	V	0.1	1	1	0.3	0.20	1
	Ni	2.5	2	2	0.6	0.10	2
	Li	2.5	25	25	7.5	0.05	25
	Sb	2.5	9	9	2.7	0.03	9
	Mo	0.3	30	30	9	0.08	30
	Cu	2.5	0.2	0.2	9	0.02	0.2
	Cr	0.8	0.5	0.5	9	0.04	0.5

Total EI contribution (µg/g)	**Cd**	**Pb**	**As**	**Hg**	**Co**	**V**	**Ni**	**Li**	**Sb**	Mo	**Cu**	**Cr**
	0.74	1.84	5.13	2.29	1.82	3.6	9.2	85.1	32.2	99.4	12.1	11.3
Concentration limit (µg/g)	0.2	0.5	1.5	0.3	0.5	1	2	25	9	150	30	110
PDE (µg/day)	2	5	15	3	5	10	20	250	90	1500	300	1100
Acceptance	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes

With the information provided in **Tables 24**, **25** and **26**, it is possible to conclude that although daily doses are more adjusted to reality, they are still very overestimated for this drug product. In this simulation was included other elements. It is important to note that has obtained for three elements the acceptance, even these values represent the worst-case scenario, but following the principals described in ICH Q3D, to validate theses calculation options it is necessary that all elements had approval. Other calculation options available in the ICH Q3D will have to be tested.

4.3.5.2. **Option 2A:** Common permitted concentration limits across drug product components for drug products with a specific daily intake:

Option 2A is similar to option 1 since it also considers that all components in the formulation contribute with the same elemental impurities amount to drug product total elemental impurity level. Distinguishing these two options is the fact that Option 2A considers that actual MDD of the drug product. MDD obtained of drug product 3 is 1.13g consequently.

Acceptance criteria for this option is similar to option 1 thus, the predicted level of elemental impurities for each component and each elemental impurity cannot be equal or superior to the calculated concentration limit.

Table 27. Predicted Elemental impurities level in drug product 3 take in consideration the option 2A.

Components		API 3	Excipient 6	Excipient 10	Excipient 2	Excipient 5	Excipient 11
Concentrations of EI (µg/g)	Cd	0.06	0.2	0.2	0.06	0.02	0.2
	Pb	0.15	0.5	0.5	0.15	0.04	0.5
	As	0.15	1.5	1.5	0.45	0.03	1.5
	Hg	0.9	0.3	0.3	0.09	0.4	0.3
	Co	0.15	0.5	0.5	0.15	0.02	0.5
	V	0.1	1	1	0.3	0.20	1
	Ni	2.5	2	2	0.6	0.10	2
	Li	2.5	25	25	7.5	0.05	25
	Sb	2.5	9	9	2.7	0.03	9
	Mo	0.3	30	30	9	0.08	30
	Cu	2.5	0.2	0.2	9	0.02	0.2
	Cr	0.8	0.5	0.5	9	0.04	0.5

Total EI contribution (µg/g)	**Cd**	**Pb**	**As**	**Hg**	**Co**	**V**	**Ni**	**Li**	**Sb**	Mo	**Cu**	**Cr**
	0.7	1.8	5.13	2.3	1.8	3.6	9.2	85.1	32.2	99.4	12.1	11.3
Concentration limit (µg/g)	1.8	4.4	13.04	2.7	4.4	8.9	17.7	221.2	79.6	1327.4	265.5	973.5
PDE (µg/day)	2	5	15	3	5	10	20	250	90	1500	300	1100
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

With the analysis of **Table 27**, it can be concluded that the total contribution of EI of each element is much lower than the CL obtained by equation 12, these elements can be used in any proportion in the drug product. This fact can be explained by the replacement of daily intake of drug product by a much more realistic scenario, MDD. Therefore, if the applicant could continue the risk analysis for the remaining sources of contamination, however, taking into account the objective of this scientific project we will continue the test of the calculation options for option 2B.

4.3.5.3. **Option 2B:** Permitted concentration limits in individual components of a product with a specified daily intake:

In option 2B are considered the quantitative composition of the drug product considering the Maximum Daily Dose of that specific product.

This option requires the determination of the maximum daily dose for each component and each element. To do this, we should multiply the predicted elemental impurities concentration value for the correspondent component daily dose (obtained by multiplying drug product MDD for the weight of the corresponding excipient).

Table 28. Predicted Elemental impurities level in drug product 3 take in consideration the option 2B.

Components:		API 3	Excipient 6	Excipient 10	Excipient 2	Excipient 11	Excipient 5
Batch Formula (mg/ml):		8.00	7.70	1.00	3.50	26.00	0.20
MDD [1] (g/day):		0.009	0.0087	0.001	0.00396	0.029	0.00023
Concentrations of EI (µg/g)	Cd	0.06	0.2	0.2	0.06	0.2	0.02
	Pb	0.15	0.5	0.5	0.15	0.5	0.04
	As	0.15	1.5	1.5	0.45	1.5	0.03
	Hg	0.9	0.3	0.3	0.09	0.3	0.4
	Co	0.15	0.5	0.5	0.15	0.5	0.02
	V	0.1	1	1	0.3	1	0.20
	Ni	2.5	2	2	0.6	2	0.10
	Li	2.5	25	25	7.5	25	0.05
	Sb	2.5	9	9	2.7	9	0.03
	Mo	0.3	30	30	9	30	0.08
	Cu	2.5	0.2	0.2	9	0.2	0.02
	Cr	0.8	0.5	0.5	9	0.5	0.04
Max daily contribution from excipient (µg/day)	Cd	0.00054	0.00174	0.0002	0.0002376	0.0058	0.0000046
	Pb	0.00135	0.00435	0.0005	0.000594	0.0145	0.0000092
	As	0.00135	0.01305	0.0015	0.001782	0.0435	0.0000069
	Hg	0.0081	0.00261	0.0003	0.0003564	0.0087	0.000092
	Co	0.00135	0.00435	0.0005	0.000594	0.0145	0.0000046
	V	0.0009	0.0087	0.001	0.001188	0.029	0.000046
	Ni	0.0225	0.0174	0.002	0.002376	0.058	0.000023
	Li	0.0225	0.2175	0.025	0.0297	0.725	0.0000115
	Sb	0.0225	0.0783	0.009	0.010692	0.261	0.0000069
	Mo	0.0027	0.261	0.03	0.03564	0.87	0.0000184
	Cu	0.0225	0.00174	0.0002	0.03564	0.0058	0.0000046
	Cr	0.0072	0.00435	0.0005	0.03564	0.0145	0.0000092

Total EI contribution (µg/day)	Cd	Pb	As	Hg	Co	V	Ni	Li	Sb	Mo	Cu	Cr
	0.009	0.02	0.06	0.02	0.02	0.04	0.1	1.02	0.38	1.19	0.066	0.062
Control threshold 30% PDE (µg/day)	0.6	1.5	4.5	0.9	1.5	3	6	75	27	450	90	330
PDE (µg/day)	2	5	15	3	5	10	20	250	90	1500	300	1100
Acceptance	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES

As shown in **Table 28**, the total daily contribution of each elemental impurities identified is much lower than the threshold 30% PDE control, which allows us to state that very large quantities would be necessary for this limit to be exceeded, although the obtained results represent a maximized scenario. The estimation of the contribution to presence of EI from Active Substance and Excipients used in formulation of API 3 eye drops solution can be obtained by option 2B.

4.3.6. Risk Assessment to Manufacturing Equipment

In pharmaceutical industry most of equipment are made of stainless steel. There are different grades of stainless steel with a range of properties, but in pharmaceutical industry the main equipment are made by austenic stainless steel series (304, 304L, 316 and 316L) because they are highly corrosion resistant and not react with the active material or excipients used in pharmaceutical manufacturing [51]. In **Table 29**, are presented the composition of grades 304, 316 and 316L.

Table 29. Composition of stainless steel grades 304, 316 and 316L. The values are present in percentage and represent the worst-case scenario.

Material	Class 1				Class 2A			Class 3					Ref.
	As	Hg	Pb	Cd	Co	Ni	V	Li	Sb	Cu	Mo	Cr	
Stainless Steel 304	NP*	NP*	NP*	NP*	NP*	12%	NP*	NP	NP*	NP*	NP*	20%	(52)
Stainless Steel 316	NP*	NP*	NP*	NP*	NP*	14%	NP*	NP	NP*	NP*	3%	18%	(53)
Stainless steel 316L	NP*	NP*	NP*	NP*	NP*	14%	NP*	NP*	NP*	NP*	3 %	18%	(53)

*NP – Not present

To realize the Risk Assessment for the manufacturing equipment, a study was carried out on the manufacturing process to identify the equipment that directly contact with the three drugs products. The identified equipment is organized in **Table 30**.

Table 30. Main equipment that directly contact with the three drugs products during their manufacturing.

Drug Product	Equipment	Brand	Material
API 1	Reactor/Mixer	Novinox 200L	Stainless Steel 316L
	Reactor/Mixer	SEITE-WERKE DB 110 A FW	Stainless Steel 316L
API 2	Reactor/Mixer	Progresso RAVS 200L	Stainless Steel 316
API 3			
All	Filling and encapsulating	IMA F57	Stainless Steel 316L

After the identification of the equipment that directly contact the products under study, it can be verified that most of them consist of 316L stainless steel, except the tubing components of filling and encapsulating machine. This machine is composed with a silicone tubes that feed the glass nozzle that dispense the correct dosage in the bottle, but in terms of contribution of these compounds they are considered **negligible**, due the time of exposure. According this, to perform the risk assessment for manufacture equipment will only take into account the constitution of stainless steel. Therefore, and take in account the information provided in **Table 29** and **30**, the composition of the stainless steel 316 and 316L is the same so, for the propose

of the risk assessment it was decided to assume the composition of the stainless steel 316L for all equipment.

To obtain information about the level of presence EI in each manufacture equipment, the manufacturers was contacted, but they only provide the analytical the quality certificates,

The Risk of EI contamination from stainless steel into the drug product during the manufacturing is raised from the passive layer, layer that forms on the surface of stainless steel and confers resistance to corrosion, not from the alloy substrate. This passive layer consists primarily of chromium oxides, hydroxides and iron compounds, which form on the outermost surface of the metal phase and the elements that may potentially leach into the drug product from passive layer are Cr, Fe, Mo and Ni (54). For the purpose of Risk Assessment was decided only include the elements that constitutes the stainless steel **(Table 29)**, Cr, Mo and Ni, so these elements are considered intentionally added.

The potential contribution of EI originating from 316L stainless steel was determined by a mathematical assay. To obtain this contribution was assumed a conservative approach, it was assumed 0.5 g/metal leaches from each produced batch to the finished drug product. This scenario was very overestimate in order to obtain an extreme scenario. This scenario was highly unlikely to happen in a GMP manufacturing site and leaching of 0.5 g/metal would be immediately detected by visual/cleaning inspection or by GMP control measures instituted. Furthermore, even 0.5 g of metal would leach from the stainless steel 316L equipment this concentration wouldn't have an impact in the finished drug product, calculations were done, to determine the level of elemental impurities derived from the stainless steel in the finished product.

To obtain the amount of metal can leach per each gram of product it was decided to use the product that had the smaller batch, which would represent the most serious scenario. Therefore, the calculations were made for the API 1 with a batch size of 130L. Assuming an estimated loss of 0.5 gram of metal per batch, per gram of product we would have 3.8 µg of metal.

It is possible to determine the corresponding concentration of Nickel, Molybdenum and Chromium per each gram of the finished drug product, considering stainless steel 316L material composition.

Ni	**Mo**	**Cr**
14%	3%	18%

$$\text{Metal/ gram of product } = 3.8\ \mu g$$

Table 31. Stainless steel 316L EI contribution in the finished drug product considering material composition.

Stainless Steel	Elemental Impurity	Calculation	Concentration (µg/g)	Parenteral PDE (µg/day)	Acceptance
316L	**Ni**	3.8 µg × 0.14	0.53	**20**	Yes
	Mo	3.8 µg × 0.03	0.11	**1500**	Yes
	Cr	3.8 µg × 0.18	0.68	**1100**	Yes

With the analysis of previous, we can conclude that the predicted concentration of elemental impurities in 316L stainless steel is much lower than the parental PDE, even though an extremely high metal concentration was assumed. Thus, an extra-large quantity of metal was necessary it was necessary to reach these limiting concentrations. The values obtained only reflects the amount of metal present in a gram of product so, if we considerate the MDD, the concentration of metals would be much lower than the value obtained. The concentration levels can be influenced by the time of residence of the solution in the equipment and by the manufacture conditions. According to the low residence times and the lack of any extremes of pH during the manufacture process we can conclude that the risk of elemental impurities inclusion from this source is **very low**.

Moreover, all equipment's are qualified and controlled by GMP's controls and according to the ICH Q3D "*Application of process knowledge, selection of equipment, equipment qualification and GMP's controls ensure a low contribution from manufacturing equipment*". Thus, given the low concentration of elemental impurities potentially derived from the manufacturing equipment, equipment qualification and GMP controls, the outcome of this assessment is that the risk of EI inclusion from manufacturing equipment is **negligible**.

4.3.7. Risk Assessment to Filters

In the pharmaceutical industry it is very common to use sterilizing filters to remove all unwanted entities from the solution without their quality being affected [(55)(56)]. During the manufacture of this three drug products, there is a sterilizing filtration stage before the solution is introduced into its primary packaging. In the sterilizing filtration there are two types of filters, polypropylene filter (PP) (Millipore Polygard CR filter) and polyvinylidene difluoride filter (PVDF) (Millipore Durapore Cartridge). Because they directly contact with the drug product can be a source of elemental impurities, so it is important to evaluate the level of elemental entities that may leachable and contaminate the drug product. In order to understand the elements that may be present in these materials, the supplier was contacted and informed that he was sensitized to this problematic, however, he did not carry out any evaluation of the presence of elemental impurities in the filters but it confirmed the absence of the elements included in class 1 of ICH Q3D, based on the information provided by the respective supplier. Nevertheless, a bibliographic search was performed to identify the main elements present in the filters.

These concentration levels, represent the amount of each elemental impurity that could be extracted under defined extraction condition. Alternatively, destructive testes, generally via digestion, can be used to quantify the level of this contaminants [3]. In most of the studies, strong acids/basis were used which, are still considerate very aggressive when compared with the conditions involved in drug product manufacturing.

Table 32. Predicted EI levels from filters materials used in the manufacture train of the drug products, obtained through extraction. Concentrations are presented in µg/g. Data retrieved from reference (3)(57).

Element		PP (Polypropylene) (µg/g)	PVDF (Polyvinylidene fluoride) (µg/g)	Total contribution of EI (µg/g)	Parenteral PDE (µg/day)	Acceptance
Class 1	Cd	≤0.01	-	≤0.01	2	Yes
	Pb	≤0.01	0.0003	≤0.0103	5	Yes
	As	≤0.01	-	≤0.01	15	Yes
	Hg	NA*	-	NA*	3	Yes
Class 2A	Co	≤0.01	0.0001	≤0.0101	5	Yes
	V	≤0.01	-	≤0.01	10	Yes
	Ni	≤0.01	0.0005	≤0.011	20	Yes
Class 2B	Se	≤0.01	-	≤0.01	80	Yes
	Ag	≤0.01	-	≤0.01	10	Yes
Class 3	Li	≤0.01	-	≤0.01	250	Yes
	Sb	≤0.01	-	≤0.01	90	Yes
	Mo	≤0.01	ND*	≤0.01	1500	Yes
	Cu	≤0.01	0.0021	≤0.0121	300	Yes
	Sn	≤0.01	0.0002	≤0.0102	600	Yes
	Cr	≤1	0.0003	≤1.0003	1100	Yes

NA* - Not Available; ND* - Not Detected

According to the information provided in **Table 32**, we can verify that the obtained values are extremely lower when compared with the PDE's, although the worse scenario has been assumed. Even so, the values obtained in the extractable tests may be overestimated because the conditions under which the tests are performed are much more aggressive than the solution properties. It is also considered that the time of exposure of the product to the filters is negligible since it is minimal. So, the potential for the filters to contaminate the drug product in significant amounts is extremely low and the predicted levels could be even lower if the maximum daily contribution had been obtained. Thus, we consider that the contribution of the filters to the presence of elemental impurities is **negligible.**

4.3.8. Risk Assessment to Water treatment system

Water is the most widely used substance, raw material or starting material in the production, processing and formulation of pharmaceutical products, so it is extremely important to control the level of contaminants by proper design of the system, periodic sanitization and by taking appropriate measures to prevent microbial proliferation. Thus, it is essential to study the entire water treatment system to identify which materials that directly contact with water and which may be potential contaminants of elemental impurities.

Water used in the manufacturing process is “Highly Purified Water” (HPW) obtained through reverse osmosis and manufacturing in situ by EDOL. In **Figure 15**, is shown a diagram of the process to obtain HPW.

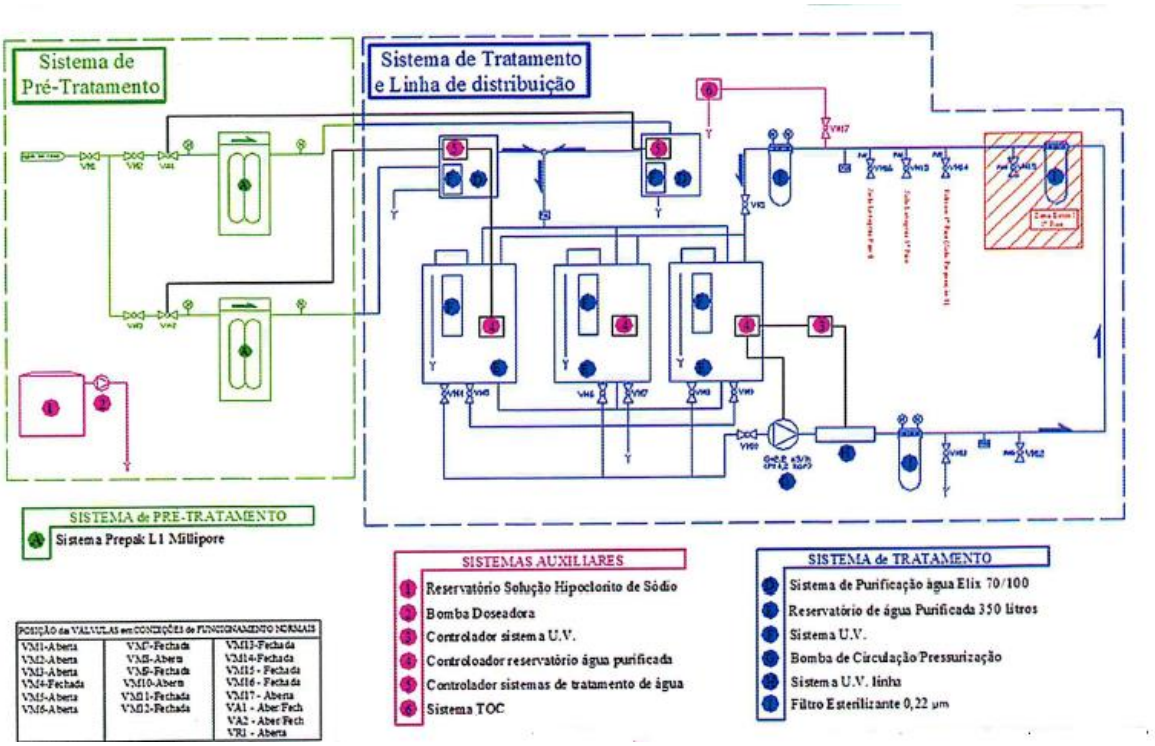

Figure 13. Diagram of the water treatment system.

The water treatment system is constituted by pre-treatment system, a treatment system and a distribution line in the form of a loop in order to obtain highly purified water. The water pre-treatment system subjects water from the public network throw a Millipore PREPACK/PROGARD particle filtering system, after this, the water is conducted by a PVC pipes to the treatment system. The treatment system consists of passing the pre-treated water through the units of reverse osmosis and eletrodeionization comprised of two units of Elix ® 70 and 100, each one equipped with UV lamp installed. The produced water coming out of these units come in three interconnected storage and distribution tanks SDS 350. All of these three tanks are equipped with a UV lamp for sanitization. In one of the distribution tanks there is a distribution pump to sending the water to the distribution loop. This line, ring-shaped, has an UV lamp, Durapore filter and a Durapore filter charged positively. After this filter there is a membrane valve, in clean area, to collect the highly purified water. The water returns to the three storage tanks after passing through another Durapore filter. Total Organic Carbon (TOC)

and the conductivity of the water distribution line is monitored on-line, using an analyser in-line.

The sanitization of the system is made whenever there is a growing trend in the level of microbial contamination but should be done at least once a year if the contamination levels are within specifications. In addition to the sanitization and maintenance, to guarantee the HPW quality, preventive physicochemical (appearance, oxidizing substances, nitrates, heavy metals, conductivity and total organic carbon (TOC)) and microbial analysis are also performed, in accordance with the European Pharmacopoeia (EP).

The water used for cleaning /rinsing equipment, containers and closure, according to GMP and “Note for guidance on Quality of Water for Pharmaceutical use (CPMP/QWP/158/01 revision)”, initial rinse for equipment, containers and closure should be purified water and in final rinse used for equipment, containers and closure should be the same quality of water used in the final stage of manufacture of the drug product.

To obtain the Risk Assessment of Water Treatment System, was necessary identify all components of this system and the respective material that composes them. All material identified are organized in **Table 33**. It is important to note that after contact with the suppliers it was not possible to obtain the material that constitutes all the components identified and thus the risk analysis will be carried out with the identified materials.

Table 33. Components of the water treatment system to be included in the Risk Assessment.

Components		Material
Transfer tubes		PVC
Particle filter Millipore PREPACK/PROGARD TL1		NA*
System Millipore Elix ® 70/ Elix ® 100	Progard TL1filter	NA*
	Pump	NA*
	Reverse Osmosis Cartridge	NA*
	Elix module	NA*
	UV lamp	Glass and Stainless Steel 316L
Storage tank Millipore SDS 350	UV lamp	Glass and Stainless Steel 316L
	Tank	NA*
Pump of storage tanks		Stainless steel 316L
Circulation/pressurizing pump Gundfos/CHI 4-50 AW-BUBE		Stainless steel 316L
UV lamp in the ring		Glass and Stainless steel 316L
Sterilizing filter Millipore Durapore charged		PVDF (Polyvinylidene fluoride) (58)
Reservoi.r of Sodium Hypochlorite solution		Polyethylene (PE)
Dosing pump Milton Roy – LMI P553-398N3		PVC
In-line analyser of TOC and Conductivity Sievers 500 RL0		NA*

NA* - Not available

The level of EI present in each component of this system was obtained by a literature, because after contact with their suppliers no answers were obtained regarding the level of this contaminants in each product. The research performed was focused on the type of material that constituted each component of the systems and the values obtained for most of the materials resulted from extraction tests carried out in conditions much more aggressive than the production of eye drops solution so, the values can be overestimated. In the case of stainless steel, which was widely used in this system, the worst-case scenario was assumed, which would be the leaching of 0.5 g of metal. The predicted elemental impurities levels from the water treatment system are shown in **Table 34**.

Table 34. Predicted EI levels from water treatment system material. Concentrations are presented in µg/g. Data retrieved from reference (53)(57)(3).

Element		PE (Polyethylene) (µg/g) (3)	Stainless Steel 316L (µg/g) (53)	PVC (Polyvinyl Chloride) (µg/g) (3)	PVDF (Polyvinylidene fluoride) (µg/g) (57)	Glass (µg/g) (3)	Total contribution of EI (µg/g)	Parenteral PDE (µg/day)	Acceptance
Class 1	Cd	≤1	NP*	≤0.01	NP*	NP*	≤1.01	2	Yes
	Pb	≤1	NP*	NP*	0.0003	≤1	≤2.0003	5	Yes
	As	≤1	NP*	≤1	NP*	≤1	≤3	15	Yes
	Hg	NP*	NP*	≤0.01	NP*	NP*	≤0.01	3	Yes
Class 2A	Co	0.01	NP*	≤0.01	0.0001	NP*	≤0.0101	5	Yes
	V	NP*	NP*	≤1	NP*	NP*	≤1	10	Yes
	Ni	NP*	1.08	≤1	0.0005	NP*	≤2.0805	20	Yes
Class 2B	Se	≤1	NP*	≤1	NP*	NP*	≤2	80	Yes
	Ag	≤1	NP*	NP*	NP*	NP*	≤1	10	Yes
Class 3	Li	≤1	NP*	NP*	NP*	NP*	≤1	250	Yes
	Sb	≤1	NP*	≤1	NP*	NP*	≤2	90	Yes
	Mo	NP*	0.20	≤1	ND*	NP*	≤1.20	1500	Yes
	Cu	≤1	NP*	≤1	0.0021	NP*	≤2.0021	300	Yes
	Sn	≤1	NP*	NP*	0.0002	NP*	≤1.0002	600	Yes
	Cr	≤ 0.01	1.2	≤0.01	0.0003	NP*	≤2.2103	1100	Yes

NP* - Not Present; ND* - Not Detected

After analysis of the **Table 34**, we can conclude that the predicted level of elemental impurities from the water treatment system are extremely low when compared with the PDE's for parenteral route. The values obtained, like we said previously are overestimate, because the concentration of some materials is obtained by extractions conditions much more aggressive than the batch conditions and for some values the concentrations obtained are much lower than the values assumed. In addition, if the maximum daily contribution of elemental impurities from water treatment system were obtained these values are much lower than the values obtained under extraction conditions. The HPW complies with the Ph.Eur. monograph and specification and quality are continuously ensured at all points of use, through frequent analysis and its risk are controlled by GMP since validation and qualification of water purification, storage and distribution systems are fundamental part of GMP and form an integral part of GMP inspection. According to ICH Q3D the risk of inclusion of elemental impurities from water can be reduced by complying with compendial Ph.Eur. water quality requirements if purified water or water for injections is used in the manufacture process.

In conclusion, even though water is considered as a potential source of elemental impurities, the implemented control system ensues that the HPW complies with the EP specifications and therefore, predicted levels of elemental impurities contribution in drug product are expected to be **negligible**. For these reasons, water will not be taken into consideration, in the remining of the Risk Assessment.

4.3.9. Risk Assessment to Container Closure System

The container closure system used for the primary packaging consists of a Low Density Polyethylene (LDPE) Bottle with a LDPE dropper dispenser. The bottle is seal with a High Density Polyethylene (HDPE) cap. The plastics used in packaging and systems delivery must be suitable with for their intended use and adequately protect the pharmaceutical product over the product's shelf-life and must be composed with materials that are safe to use, because some interactions between a plastic material and the pharmaceutical product, like sorption, uptake of product components by the plastic material, leaching, release of plastic material components to the product, can affect the quality of drug product (59)(60).

To evaluate the levels of elemental impurities that can leaching from the container closure system to the drug product, was contacted the manufacturer of the container closure system. He informed that he had contacted his suppliers and had been informed by the supplier of the resins that any elements included in class 1, 2, 3 listed in ICH Q3D are not intentionally added during the manufacture of resins. More inform, that the manufacturer of the Remafin white informs that the elements As, Cd, Pb, Hg, Cr, Cu, Ni, Ru, V, Sb, Ba, Co, Se and Sn have not ether intentionally added during its production. It is important to refer that all standard white products are tested to poof the compliance with the USP chapter <661> and EP chapter <3.1.5>.

Risk Assessment was performed for the components of the container closure system which directly contacts with the drug product. Therefore, this was only done for LDPE since it is considered that the product is only in contact with the bottle and with the dropper dispenser. The elements included in this Risk Evaluation are those required by ICH Q3D guideline for parental route which assumed the values obtained by the USP chapter <661>. In **Table 35** are presented the predicted elemental impurities levels from the LDPE bottle and dropper dispenser into drug product.

Table 35. Predicted EI levels from LDPE bottle and dropper dispenser. Concentrations are presented in µg/g.

Material	Elemental Impurities	Concentration of EI (µg/g)	Parental PDE (µg/day)	Acceptance
Low Density Polyethylene (LDPE)	**Cd**	<0.2	2	Yes
	Pb	<0.2	5	Yes
	As	<0.2	15	Yes
	Hg	<0.2	3	Yes
	Co	-	5	-
	V	-	10	-
	Ni	-	20	-
	Li	-	250	-
	Sb	<0.2	90	Yes
	Cu	<0.2	300	Yes

It is important to refer that the concentration of elemental impurities obtained from USP <661> represent the amount of metals present in the LDPE plastic, so for the purposes of the Risk

Assessment it was assumed that the levels of elemental impurities obtained by USP <661> are totally leached to the drug product during it shelf-time. Even assuming this scenario, the concentration of elemental impurities is well below than the limiting concentrations. These values can be much lower if the maximum daily contribution of the LDPE were obtained. Therefore, we can affirm that the liberation from the packaging components represents an essentially low safety risk. Thus, taking into account the available results we can affirm that the contribution of the packaging components to the elemental impurities in eye drops solution is considered **negligible**.

4.3.10. Summary of the Risk Assessment

In this summary of the Risk Assessment performed all relevant sources of potential EI have been identified and tested using a component approach with the scope to identify the contribution of each source to the presence of EI in final drug product. To do this, all information regarding to the EI levels is based on data provided by the manufacturers or based on data obtained to the scientific literature. It´s important to refer that in both cases the values obtained are overestimate, allowing to obtain an extreme scenario. To perform this summary of the Risk Assessment, was tested some approaches to get the best way to perform this Risk Assessment. Therefore, since the contribution of the Active Substance and Excipients that compound this drug product passed in calculation options 2A and 2B, three scenarios were tested for these two calculation options:

- The predicted max contribution for each EI is the highest value observed in all components included in the Risk Assessment.
- The predicted max contribution for each EI is the sum of the contribution of each component included in the Risk Assessment.
- The predicted max contribution for each EI is the sum of each component of the Risk Assessment which hasn’t considered **negligible**.

4.3.10.1. Summary of Risk Assessment for drug product 1.

Table 36. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2A**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.88	No	≤ 0.01	≤1.01	No	≤1.01	0.6	**YES**
Pb	1	2.19	No	≤ 0.0103	≤2.00	<0.2	≤2.19	1.5	**YES**
As	1	6.48	No	≤ 0.01	≤3	<0.2	≤6.48	4.5	**YES**
Hg	1	1.69	No	NA*	≤0.01	<0.2	≤1.69	0.9	**YES**
Co	2A	2.17	No	≤ 0.01	≤0.01	No	≤2.17	1.5	**YES**
V	2A	4.5	No	≤ 0.01	≤1	No	≤4.5	3	**YES**
Ni	2A	8.7	0.53	≤ 0.011	≤2.08	No	≤8.7	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	107.55	No	≤ 0.01	≤1	No	≤107.5	75	**YES**
Sb	3	167.81	No	≤ 0.01	≤2	<0.2	≤167.8	27	**YES**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤1.20	450	**No controls required**
Cu	3	129.08	No	≤ 0.012	≤2.012	<0.2	≤129.08	90	**YES**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤1.00	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤2.21	330	**No controls required**

Table 37. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2B**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.001	No	≤ 0.01	≤1.01	No	≤1.01	0.6	**YES**
Pb	1	0.0027	No	≤ 0.0103	≤2.00	<0.2	≤2.0	1.5	**YES**
As	1	0.008	No	≤ 0.01	≤3	<0.2	≤3.00	4.5	**No controls required**
Hg	1	0.002	No	NA*	≤0.01	<0.2	≤0.2	0.9	**No controls required**
Co	2A	0.003	No	≤ 0.01	≤0.01	No	0.02	1.5	**No controls required**
V	2A	0.005	No	≤ 0.01	≤1	No	≤1.00	3	**No controls required**
Ni	2A	0.01	0.53	≤ 0.011	≤2.08	No	≤2.08	6	**No controls required**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	0.135	No	≤ 0.01	≤1	No	≤1.00	75	**No controls required**
Sb	3	0.067	No	≤ 0.01	≤2	<0.2	≤2.00	27	**No controls required**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤1.20	450	**No controls required**
Cu	3	0.162	No	≤ 0.012	≤2.012	<0.2	≤2.00	90	**No controls required**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤1.00	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤2.21	330	**No controls required**

NA*- Not available

Table 38. Conclusion summary of EI Risk Assessment assuming the contribution of Active Substance and Excipients given by **option 2A** and all contribution given by all components of the Risk Assessment against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.88	No	≤ 0.01	≤1.01	No	≤ 1.9	0.6	**YES**
Pb	1	2.19	No	≤ 0.0103	≤2.00	<0.2	≤ 4.4003	1.5	**YES**
As	1	6.48	No	≤ 0.01	≤3	<0.2	≤ 9.49	4.5	**YES**
Hg	1	1.69	No	NA*	≤0.01	<0.2	≤ 1.7	0.9	**YES**
Co	2A	2.17	No	≤ 0.01	≤0.01	No	≤ 2.19	1.5	**YES**
V	2A	4.5	No	≤ 0.01	≤1	No	≤ 5.51	3	**YES**
Ni	2A	8.7	0.53	≤ 0.011	≤2.08	No	≤ 11.321	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤ 2.01	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤ 1.21	3	**No controls required**
Li	2B	107.55	No	≤ 0.01	≤1	No	≤ 108.56	75	**YES**
Sb	3	167.81	No	≤ 0.01	≤2	<0.2	≤ 170.02	27	**YES**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤ 1.31	450	**No controls required**
Cu	3	129.08	No	≤ 0.012	≤2.012	<0.2	≤130.1	90	**YES**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤ 1.0104	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤ 2.89	330	**No controls required**

NA*- Not available

Table 39. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2A** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.88	No	Negligible risk	Negligible risk	No	0.88	0.6	**YES**
Pb	1	2.19	No	Negligible risk	Negligible risk	Negligible risk	2.19	1.5	**YES**
As	1	6.48	No	Negligible risk	Negligible risk	Negligible risk	6.48	4.5	**YES**
Hg	1	1.69	No	NA*	Negligible risk	Negligible risk	1.69	0.9	**YES**
Co	2A	2.17	No	Negligible risk	Negligible risk	No	2.17	1.5	**YES**
V	2A	4.5	No	Negligible risk	Negligible risk	No	4.5	3	**YES**
Ni	2A	8.7	Negligible risk	Negligible risk	Negligible risk	No	8.7	6	**YES**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	107.55	No	Negligible risk	Negligible risk	No	107.55	75	**YES**
Sb	3	167.81	No	Negligible risk	Negligible risk	Negligible risk	167.81	27	**YES**
Mo	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	450	**-**
Cu	3	129.08	No	Negligible risk	Negligible risk	Negligible risk	129.08	90	**YES**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	330	**-**

Table 40. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2B** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/day)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment	Filters	Water Treatment System	Container Closure System			
Cd	1	0.008	No	Negligible risk	Negligible risk	No	0.008	0.6	**No controls required**
Pb	1	0.02	No	Negligible risk	Negligible risk	Negligible risk	0.02	1.5	**No controls required**
As	1	0.06	No	Negligible risk	Negligible risk	Negligible risk	0.06	4.5	**No controls required**
Hg	1	0.01	No	NA*	Negligible risk	Negligible risk	0.01	0.9	**No controls required**
Co	2A	0.02	No	Negligible risk	Negligible risk	No	0.02	1.5	**No controls required**
V	2A	0.04	No	Negligible risk	Negligible risk	No	0.04	3	**No controls required**
Ni	2A	0.08	Negligible risk	Negligible risk	Negligible risk	No	0.08	6	**No controls required**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	1.0	No	Negligible risk	Negligible risk	No	1.0	75	**No controls required**
Sb	3	0.37	No	Negligible risk	Negligible risk	Negligible risk	0.37	27	**No controls required**
Mo	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	450	**-**
Cu	3	1.25	No	Negligible risk	Negligible risk	Negligible risk	1.25	90	**No controls required**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	330	**-**

NA*- Not available

4.3.10.2. Summary of Risk Assessment for drug product 2.

Table 41. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2A**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	1.08	No	≤ 0.01	≤1.01	0.2	≤1.08	0.6	**YES**
Pb	1	2.69	No	≤ 0.0103	≤2.00	<0.2	≤2.69	1.5	**YES**
As	1	7.98	No	≤ 0.01	≤3	<0.2	≤7.98	4.5	**YES**
Hg	1	1.99	No	NA*	≤0.01	<0.2	≤1.99	0.9	**YES**
Co	2A	2.67	No	≤ 0.01	≤0.01	No	≤2.67	1.5	**YES**
V	2A	5.5	No	≤ 0.01	≤1	No	≤5.5	3	**YES**
Ni	2A	10.7	0.53	≤ 0.011	≤2.08	No	≤10.7	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	132.55	No	≤ 0.01	≤1	No	≤132.55	75	**YES**
Sb	3	47.73	No	≤ 0.01	≤2	<0.2	≤47.73	27	**YES**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤1.20	450	**No controls required**
Cu	3	159.1	No	≤ 0.012	≤2.012	<0.2	≤159.1	90	**YES**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤1.00	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤2.21	330	**No controls required**

NA*- Not available

Table 42. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2B**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.001	No	≤ 0.01	≤1.01	0.2	≤1.01	0.6	**YES**
Pb	1	0.003	No	≤ 0.0103	≤2.00	<0.2	≤2.0	1.5	**YES**
As	1	0.009	No	≤ 0.01	≤3	<0.2	≤3.00	4.5	**No controls required**
Hg	1	0.002	No	NA*	≤0.01	<0.2	≤0.2	0.9	**No controls required**
Co	2A	0.003	No	≤ 0.01	≤0.01	No	≤0.0101	1.5	**No controls required**
V	2A	0.006	No	≤ 0.01	≤1	No	≤1.00	3	**No controls required**
Ni	2A	0.012	0.53	≤ 0.011	≤2.08	No	≤2.0805	6	**No controls required**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	0.145	No	≤ 0.01	≤1	No	≤1.00	75	**No controls required**
Sb	3	0.052	No	≤ 0.01	≤2	<0.2	≤2.00	27	**No controls required**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤1.20	450	**No controls required**
Cu	3	0.174	No	≤ 0.012	≤2.012	<0.2	≤2.0021	90	**No controls required**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤1.00	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤2.21	330	**No controls required**

NA*- Not available

Table 43. Conclusion summary of EI Risk Assessment assuming the contribution of Active Substance and Excipients given by **option 2A** and all contribution given by all components of the Risk Assessment against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	1.08	No	≤ 0.01	≤1.01	0.2	≤2.3	0.6	**YES**
Pb	1	2.69	No	≤ 0.0103	≤2.00	<0.2	≤7.0003	1.5	**YES**
As	1	7.98	No	≤ 0.01	≤3	<0.2	≤11.19	4.5	**YES**
Hg	1	1.99	No	NA*	≤0.01	<0.2	≤2.2	0.9	**YES**
Co	2A	2.67	No	≤ 0.01	≤0.01	No	≤2.69	1.5	**YES**
V	2A	5.5	No	≤ 0.01	≤1	No	≤6.51	3	**YES**
Ni	2A	10.7	0.53	≤ 0.011	≤2.08	No	≤13.3	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.01	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.21	3	**No controls required**
Li	2B	132.55	No	≤ 0.01	≤1	No	≤133.56	75	**YES**
Sb	3	47.73	No	≤ 0.01	≤2	<0.2	≤49.94	27	**YES**
Mo	3	No	0.11	≤ 0.01	≤1.20	No	≤1.32	450	**No controls required**
Cu	3	159.1	No	≤ 0.012	≤2.012	<0.2	≤162.63	90	**YES**
Sn	3	No	No	≤ 0.0102	≤1.0002	No	≤1.0104	180	**No controls required**
Cr	3	No	0.68	≤ 0.0003	≤2.2103	No	≤2.81	330	**No controls required**

NA*- Not available

Table 44. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2A** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	1.08	No	Negligible risk	Negligible risk	Negligible risk	1.08	0.6	**YES**
Pb	1	2.69	No	Negligible risk	Negligible risk	Negligible risk	2.69	1.5	**YES**
As	1	7.98	No	Negligible risk	Negligible risk	Negligible risk	7.98	4.5	**YES**
Hg	1	1.99	No	NA*	Negligible risk	Negligible risk	1.99	0.9	**YES**
Co	2A	2.67	No	Negligible risk	Negligible risk	No	2.67	1.5	**YES**
V	2A	5.5	No	Negligible risk	Negligible risk	No	5.5	3	**YES**
Ni	2A	10.7	Negligible risk	Negligible risk	Negligible risk	No	10.7	6	**YES**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	132.55	No	Negligible risk	Negligible risk	No	132.55	75	**YES**
Sb	3	47.73	No	Negligible risk	Negligible risk	Negligible risk	47.73	27	**YES**
Mo	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	450	**-**
Cu	3	159.1	No	Negligible risk	Negligible risk	Negligible risk	159.1	90	**YES**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	330	**-**

NA*- Not available

Table 45. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2B** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/day)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment	Filters	Water Treatment System	Container Closure System			
Cd	1	0.001	No	Negligible risk	Negligible risk	Negligible risk	0.001	0.6	**No controls required**
Pb	1	0.003	No	Negligible risk	Negligible risk	Negligible risk	0.003	1.5	**No controls required**
As	1	0.009	No	Negligible risk	Negligible risk	Negligible risk	0.009	4.5	**No controls required**
Hg	1	0.002	No	NA*	Negligible risk	Negligible risk	0.002	0.9	**No controls required**
Co	2A	0.003	No	Negligible risk	Negligible risk	No	0.003	1.5	**No controls required**
V	2A	0.006	No	Negligible risk	Negligible risk	No	0.006	3	**No controls required**
Ni	2A	0.012	Negligible risk	Negligible risk	Negligible risk	No	0.012	6	**No controls required**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	0.145	No	Negligible risk	Negligible risk	No	0.145	75	**No controls required**
Sb	3	0.052	No	Negligible risk	Negligible risk	Negligible risk	0.052	27	**No controls required**
Mo	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	450	**-**
Cu	3	0.174	No	Negligible risk	Negligible risk	Negligible risk	0.174	90	**No controls required**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	No	Negligible risk	Negligible risk	Negligible risk	No	-	330	**-**

NA*- Not available

4.3.10.3. Summary of Risk Assessment for drug product 3.

Table 46. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2A**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients	Manufacturing Equipment	Filters	Water Treatment System	Container Closure System			
Cd	1	0.7	No	≤ 0.01	≤1.01	<0.2	≤1.08	0.6	**YES**
Pb	1	1.8	No	≤ 0.01	≤2.00	<0.2	≤2.0003	1.5	**YES**
As	1	5.13	No	≤ 0.01	≤3	<0.2	≤5.13	4.5	**YES**
Hg	1	2.3	No	NA*	≤0.01	<0.2	≤2.3	0.9	**YES**
Co	2A	1.8	No	≤ 0.01	≤0.01	No	≤1.8	1.5	**YES**
V	2A	3.6	No	≤ 0.01	≤1	No	≤3.6	3	**YES**
Ni	2A	9.2	0.53	≤ 0.01	≤2.08	No	≤9.2	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	85.1	No	≤ 0.01	≤1	No	≤85.1	75	**YES**
Sb	3	32.2	No	≤ 0.01	≤2	<0.2	≤32.2	27	**YES**
Mo	3	99.4	0.11	≤ 0.01	≤1.20	No	≤99.4	450	**No controls required**
Cu	3	12.1	No	≤ 0.01	≤2.01	<0.2	≤12.1	90	**No controls required**
Sn	3	No	No	≤ 0.01	≤1.00	No	≤1.0002	180	**No controls required**
Cr	3	11.3	0.68	≤ 0.0003	≤2.21	No	≤11.3	330	**No controls required**

NA* - Not Available

Table 47. Conclusion summary of EI Risk Assessment assuming the highest value of each element observed in all Risk Assessment components against the Control Threshold for each identified elemental impurity. Contribution of drug substance and excipients by **Option 2B**.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.009	No	≤ 0.01	≤1.01	<0.2	≤1.01	0.6	**YES**
Pb	1	0.02	No	≤ 0.01	≤2.00	<0.2	≤2.00	1.5	**YES**
As	1	0.06	No	≤ 0.01	≤3	<0.2	≤3.00	4.5	**No controls required**
Hg	1	0.02	No	NA*	≤0.01	<0.2	≤0.2	0.9	**No controls required**
Co	2A	0.02	No	≤ 0.01	≤0.01	No	≤0.02	1.5	**No controls required**
V	2A	0.04	No	≤ 0.01	≤1	No	≤1.00	3	**No controls required**
Ni	2A	0.1	0.53	≤ 0.01	≤2.08	No	≤2.08	6	**No controls required**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.00	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.00	3	**No controls required**
Li	2B	1.02	No	≤ 0.01	≤1	No	≤1.00	75	**No controls required**
Sb	3	0.38	No	≤ 0.01	≤2	<0.2	≤2.00	27	**No controls required**
Mo	3	1.19	0.11	≤ 0.01	≤1.20	No	≤1.20	450	**No controls required**
Cu	3	0.07	No	≤ 0.01	≤2.01	<0.2	≤2.01	90	**No controls required**
Sn	3	No	No	≤ 0.01	≤1.00	No	≤1.00	180	**No controls required**
Cr	3	0.06	0.68	≤ 0.0003	≤2.21	No	≤2.21	330	**No controls required**

NA*- Not available

Table 48. Conclusion summary of EI Risk Assessment assuming the contribution of Active Substance and Excipients given by **option 2A** and all contribution given by all components of the Risk Assessment against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE (µg/day)	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.7	No	≤ 0.01	≤1.01	<0.2	≤1.92	0.6	**YES**
Pb	1	1.8	No	≤ 0.01	≤2.00	<0.2	≤4.01	1.5	**YES**
As	1	5.13	No	≤ 0.01	≤3	<0.2	≤8.34	4.5	**YES**
Hg	1	2.3	No	NA*	≤0.01	<0.2	≤2.51	0.9	**YES**
Co	2A	1.8	No	≤ 0.01	≤0.01	No	≤1.82	1.5	**YES**
V	2A	3.6	No	≤ 0.01	≤1	No	≤4.61	3	**YES**
Ni	2A	9.2	0.53	≤ 0.01	≤2.08	No	≤11.82	6	**YES**
Se	2B	No	No	≤ 0.01	≤2	No	≤2.01	130	**No controls required**
Ag	2B	No	No	≤ 0.01	≤1	<0.2	≤1.21	3	**No controls required**
Li	2B	85.1	No	≤ 0.01	≤1	No	≤86.11	75	**YES**
Sb	3	32.2	No	≤ 0.01	≤2	<0.2	≤34.41	27	**YES**
Mo	3	99.4	0.11	≤ 0.01	≤1.20	No	≤100.72	450	**No controls required**
Cu	3	12.1	No	≤ 0.01	≤2.01	<0.2	≤13.11	90	**No controls required**
Sn	3	No	No	≤ 0.01	≤1.00	No	≤1.01	180	**No controls required**
Cr	3	11.3	0.68	≤ 0.0003	≤2.21	No	≤14.19	330	**No controls required**

NA*- Not available

Table 49. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2A** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/g)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/g)	Manufacturing Equipment (µg/g)	Filters (µg/g)	Water Treatment System (µg/g)	Container Closure System (µg/g)			
Cd	1	0.7	No	Negligible risk	Negligible risk	Negligible risk	0.7	0.6	**YES**
Pb	1	1.8	No	Negligible risk	Negligible risk	Negligible risk	1.8	1.5	**YES**
As	1	5.13	No	Negligible risk	Negligible risk	Negligible risk	5.13	4.5	**YES**
Hg	1	2.3	No	NA*	Negligible risk	Negligible risk	2.3	0.9	**YES**
Co	2A	1.8	No	Negligible risk	Negligible risk	No	1.8	1.5	**YES**
V	2A	3.6	No	Negligible risk	Negligible risk	No	3.6	3	**YES**
Ni	2A	9.2	Negligible risk	Negligible risk	Negligible risk	No	9.3	6	**YES**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	85.1	No	Negligible risk	Negligible risk	No	85.1	75	**YES**
Sb	3	32.2	No	Negligible risk	Negligible risk	Negligible risk	32.2	27	**YES**
Mo	3	99.4	Negligible risk	Negligible risk	Negligible risk	No	99.4	450	**No controls required**
Cu	3	12.1	No	Negligible risk	Negligible risk	Negligible risk	12.1	90	**No controls required**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	11.3	Negligible risk	Negligible risk	Negligible risk	No	11.3	330	**No controls required**

Table 50. Conclusion summary of EI Risk Assessment obtained only by the contribution of Active Substance and Excipients given by **option 2B** against the Control Threshold for each identified elemental impurity.

Element	Class	Presence/Risk					Max predicted EI exposure (µg/day)	Control Threshold 30% of Parental PDE	Actions/Control Strategy
		Drug Substance and Excipients (µg/day)	Manufacturing Equipment	Filters	Water Treatment System	Container Closure System			
Cd	1	0.009	No	Negligible risk	Negligible risk	Negligible risk	0.009	0.6	**No controls required**
Pb	1	0.02	No	Negligible risk	Negligible risk	Negligible risk	0.02	1.5	**No controls required**
As	1	0.06	No	Negligible risk	Negligible risk	Negligible risk	0.06	4.5	**No controls required**
Hg	1	0.02	No	NA*	Negligible risk	Negligible risk	0.02	0.9	**No controls required**
Co	2A	0.02	No	Negligible risk	Negligible risk	No	0.02	1.5	**No controls required**
V	2A	0.04	No	Negligible risk	Negligible risk	No	0.04	3	**No controls required**
Ni	2A	0.1	Negligible risk	Negligible risk	Negligible risk	No	0.1	6	**No controls required**
Se	2B	No	No	Negligible risk	Negligible risk	No	-	130	**-**
Ag	2B	No	No	Negligible risk	Negligible risk	Negligible risk	-	3	**-**
Li	2B	1.02	No	Negligible risk	Negligible risk	No	1.02	75	**No controls required**
Sb	3	0.38	No	Negligible risk	Negligible risk	Negligible risk	0.38	27	**No controls required**
Mo	3	1.19	Negligible risk	Negligible risk	Negligible risk	No	1.19	450	**No controls required**
Cu	3	0.07	No	Negligible risk	Negligible risk	Negligible risk	0.07	90	**No controls required**
Sn	3	No	No	Negligible risk	Negligible risk	No	-	180	**-**
Cr	3	0.06	Negligible risk	Negligible risk	Negligible risk	No	0.06	330	**No controls required**

NA*- Not available

4.3.11. Discussion of Results

In this chapter we intend to discuss all the results obtained through the implementation of the methodology presented in the section: "4.1.1.1 Methodology used to perform the Risk Assessment".

As previously mentioned during this scientific project, the lack of information negatively conditioned the results obtained, since the concentrations assumed for the elements that did not have information are maximized. This factor may be at the origin of the reprobation of the calculation option 1 in the evaluation of the contribution of the active substance and excipients. In the remaining options, 2A and 2B, the approval was obtained, although in option 2A the concentrations are inferior to the obtained CLs.

Regarding the contribution of the other sources of elemental impurities identified, it is important to mention that the list of materials that come into contact with the product could be more exhaustive, however, due to the difficulty in obtaining information of the constitution of each equipment, it was decided to assuming a simplified scenario taking the worst-case scenario. This scenario resulting in very high EI concentrations. Nevertheless, these levels were below the parenteral PDE, leading to their approval and consequently, following the principles described in ICH Q3D, excluded from the Risk Assessment. However, in this scientific project several scenarios were studied to obtain the summary of the Risk Assessment.

The approaches under study are described in the section "4.1.10 Summary of the Risk Assessment" and the results obtained are organized in tables. In the first approach **(Tables 36, 41 and 46)** are presented the result of the Risk Assessment from assuming the maximum concentration of each EI obtained from all possible sources of contamination. It was assumed either that the contribution of the active substance and excipients is given by the calculation option 2A. With the analysis of this table, we can verify that for most EI, it is necessary to adopt control actions, since they exceed 30% of the parenteral PDE. For all products, this problem can be justified with the high concentration values obtained by calculation option 2A, although the concentrations of each impurity are below the CL obtained. This high concentrations values are related with the overestimated scenario used to estimate the contribution of each compound. On the other hand, the results obtained by assuming the daily contribution of each elemental impurity **(Tables 36, 42 and 47)** are really, most EI had approval and control actions are not required, only for Cd and Pb.

In **Tables 38, 43** and **48** are the results obtained from the sum of the contribution of all the sources considered in the Risk Assessment. It should be noted that this study was only possible for the calculation option 2A, since the contributions of each component were expressed in µg/g and in option 2B the results are in µg/day. Therefore, this scenario is unlikely to happen, because most of EI exceed the control threshold 30% of the parental PDE and consequently additional measures needs to be applied to control the levels of EI in final drug product. It is important to note that this result can be affected by the assumptions that a very high amount of the EI migrate to the drug product, since the extractions tests obtained by bibliographic search are carried out under much more aggressive conditions of extraction when compared with the conditions of the drug product.

The third approach consists of excluding all potential considered **negligible** of the Risk Assessment and the others are sum to obtain the max EI contribution, the application of this approach are placed in **Tables 39**, **40**, **44**, **45**, **49** and **50**. Comparing the results of this two tables it is possible to verify that the best results are obtained with option 2B, where all EI have acceptance.

Thus, in view of the results obtained previously, the best way to obtain the Risk assessment is to choose the third approach conjugated with the 2B calculation option. However, it is believed that possibly the second approach would work with 2B calculation option only if the daily contribution of all the participants in the Risk Assessment had been obtained.

CHAPTER 5 – Conclusion

With this research it was possible to verify that EI are commonly present in most of the materials that directly contact with the drug product and may affect the efficacy of the drug and the health of the patient.

The lack of data is a great constraint for the realization of the Risk Assessment, as it is verified in the present scientific project in which it was necessary to recourse to bibliographical research to assume the worst case, since the data obtained from extractables corresponded to conditions much more aggressive than those offered by the product itself during its production.

It has also been verified that the three test products, despite being used in quite different dosage regimens, are quite similar in terms of raw materials used in their production, manufacture train and packaging systems. Therefore, the methodologies applied, and the results obtained are similar, so there is no relationship between the mode of administration of the drug and the presence of EI.

This guide presents some limitations in particular in identifying the sources of elemental impurities, where people and methods can have some impact, and in calculating options. During the simulation of the calculation options, that in all the products none obtained approval in option 1. In this scientific project it was tried to readjust the daily dose of drug product taking into account the typologies of existing systems of amazement however, taking into account the results obtained these still represent rather overrated scenario. Therefore, it would be advisable to readjust this formula for more adequate values, taking into account the route of administration and the pharmaceutical form.

Based on the results obtained, it is concluded that the best calculation option to estimate the contribution of Active Substance and Excipients will be 2B, however this option implies the daily quantification of the contribution of each of the sources, which is a challenge given the scarcity of data.

Concluding, we can affirm that in the present scientific project it presents an adequate methodology for the quantification of EI in the final product, however, in view of the condition presented previously, a very maximized approach is obtained. Given these facts and in the presence of more adequate EI values, it is possible to affirm that the results obtained would be very different in all the approaches assumed during this project.

CHAPTER 6 – Further Considerations

In order to have continuity of the present project, it would be interesting to explore the best calculation option obtained in this project (2B) since for this to happen it is necessary to obtain the daily amounts that each of the possible sources of contamination contributes.

It would still be interesting to apply the QRM principles, as described in ICH Q9.

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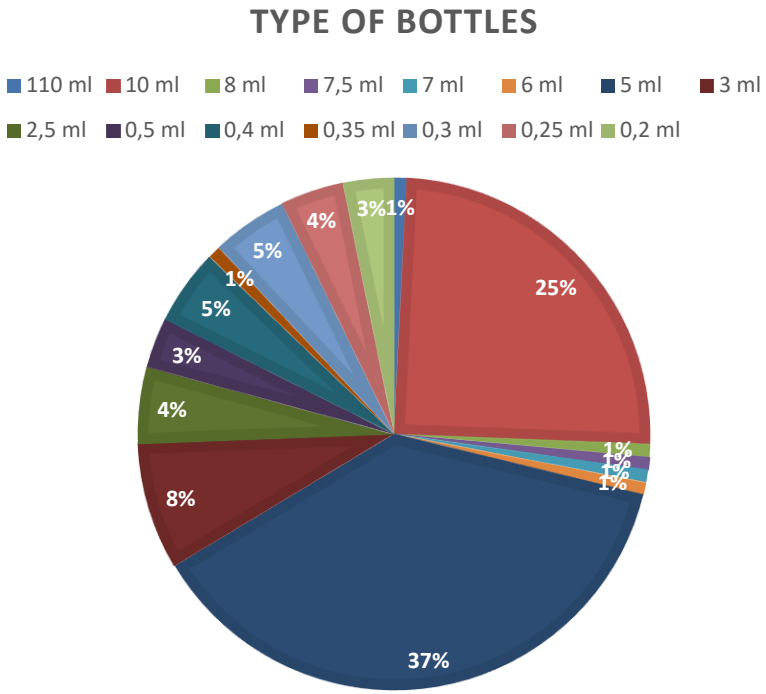
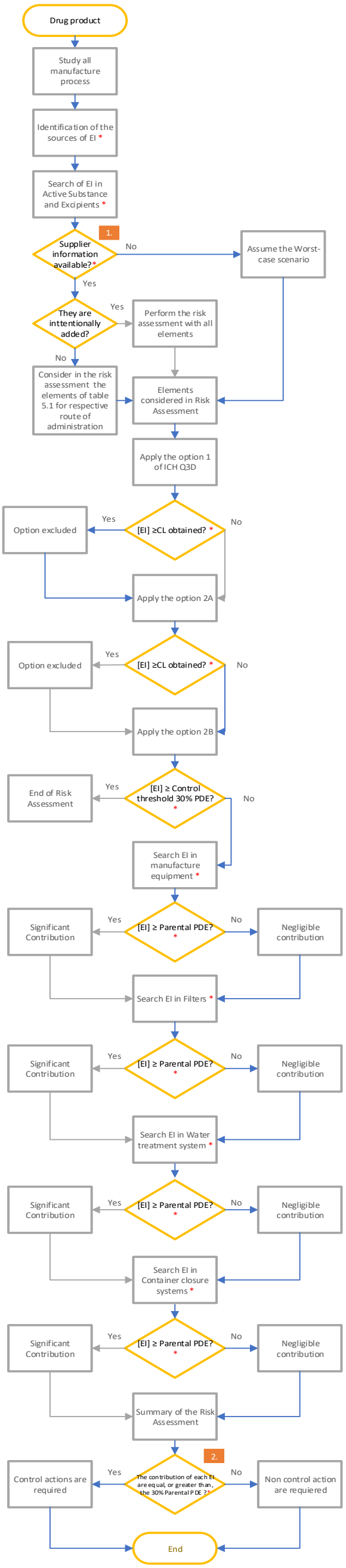
Appendix 1

According to the database provided by Infarmed, we obtained this circular diagram that allows us to obtain the most common types of bottles for eye drops solution.



Appendix 2 – Risk Assessment Methodology



Risk Assessment Legend:

- Decision taken
- Steps of the Risk Assessment
- * Critical points